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Enoki et al.

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(45) **Date of Patent: Dec. 24, 2019**

(54) **MARKER ASSOCIATED WITH RESISTANCE TO SMUT IN PLANT BELONGING TO GENUS *SACCHARUM*, AND USE THEREOF**

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(73) Assignee: **TOYOTA JIDOSHA KABUSHIKI KAISHA**, Toyota-shi, Aichi-ken (JP)

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Related U.S. Application Data

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(30) **Foreign Application Priority Data**

Apr. 28, 2011 (JP) 2011-101050
Apr. 18, 2012 (JP) 2012-094995

(51) **Int. Cl.**

C12Q 1/68	(2018.01)
A01H 1/04	(2006.01)
C12Q 1/6895	(2018.01)
A01H 5/04	(2018.01)

(52) **U.S. Cl.**

CPC ***C12Q 1/6895*** (2013.01); ***A01H 1/04*** (2013.01); ***A01H 5/04*** (2013.01); *C12Q 2600/13* (2013.01); *C12Q 2600/156* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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Final Office Action, dated May 3, 2016, issued by the United States Patent and Trademark Office in U.S. Appl. No. 14/113,539.

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(Continued)

Primary Examiner — Bratislav Stankovic

(74) *Attorney, Agent, or Firm* — Sughrue Mion, PLLC

(57) **ABSTRACT**

The present invention relates to a marker associated with resistance to smut which is a quantitative trait of sugarcane. Specifically, a marker associated with resistance to sugarcane smut, which consists of a continuous nucleic acid region existing in a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 1 and the nucleotide sequence shown in SEQ ID NO: 14 or a different similar region, is provided.

4 Claims, 19 Drawing Sheets

Specification includes a Sequence Listing.

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Communication, dated Mar. 15, 2019, issued by the United States Patent and Trademark Office in U.S. Appl. No. 15/664,114.
Communication, dated Aug. 20, 2019, issued by the United States Patent and Trademark Office in U.S. Appl. No. 15/664,139.
Communication, dated Aug. 1, 2019, issued by the United States Patent and Trademark Office in U.S. Appl. No. 15/664,114.

* cited by examiner

Fig. 1

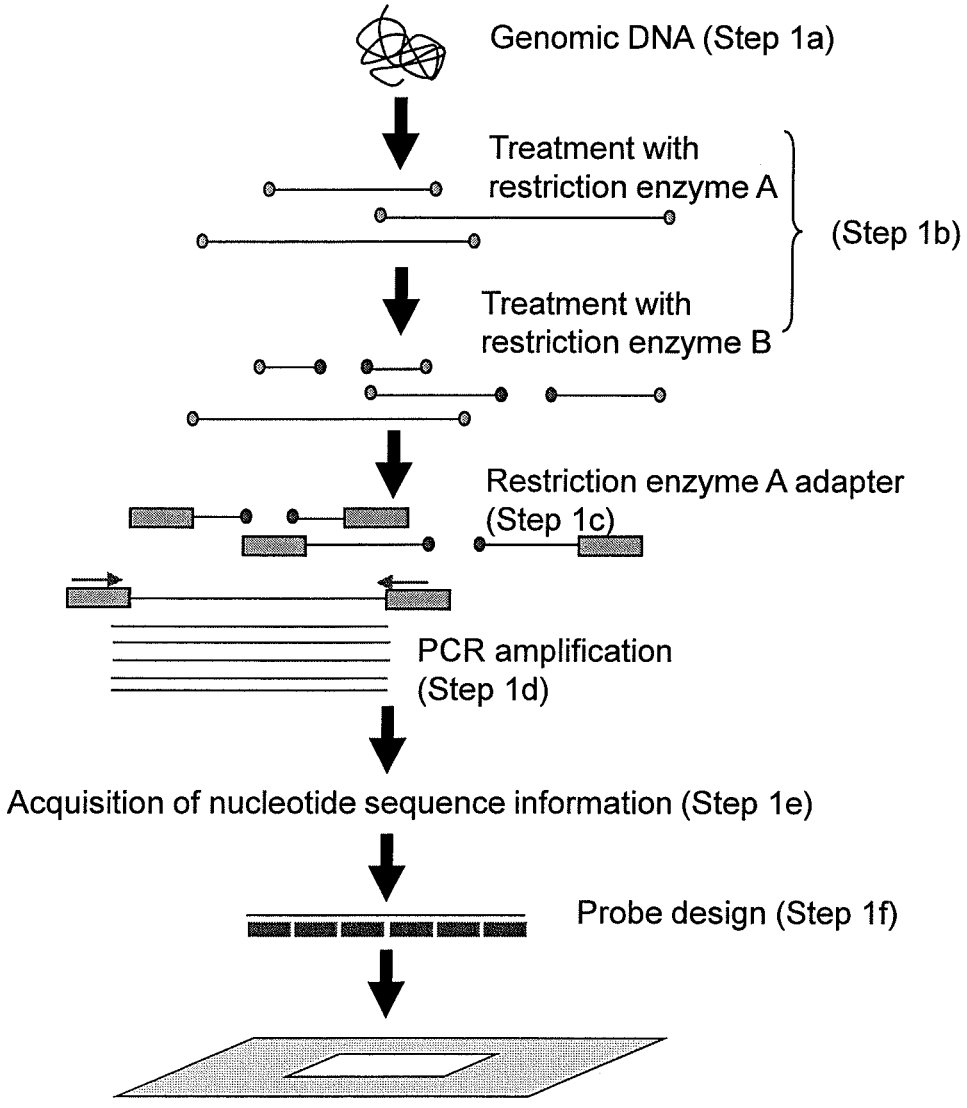

Genomic DNA (Step 1a)
Treatment with restriction enzyme A
(Step 1b)
Treatment with restriction enzyme B
Restriction enzyme A adapter (Step 1c)
PCR amplification (Step 1d)
Acquisition of nucleotide sequence information (Step 1e)
Probe design (Step 1f)

Fig. 2

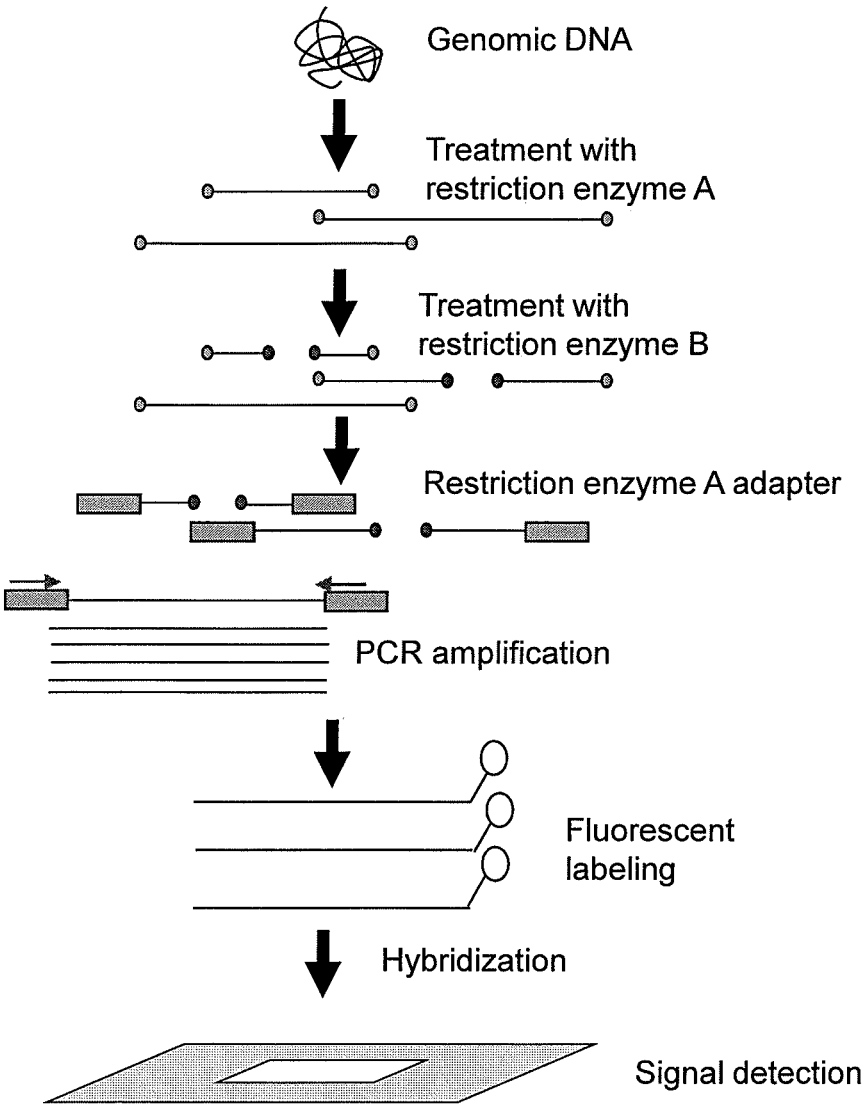
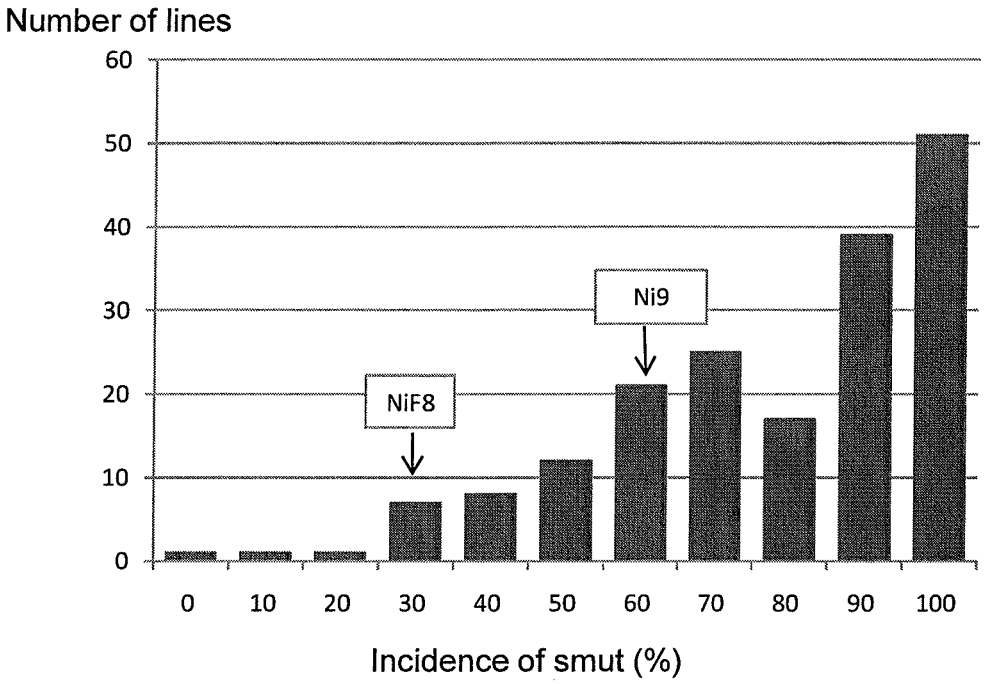

Genomic DNA
Treatment with
restriction enzyme A
Treatment with
restriction enzyme B
Restriction enzyme A adapter
PCR amplification
Fluorescent
labeling
Hybridization
Signal detection

Fig. 3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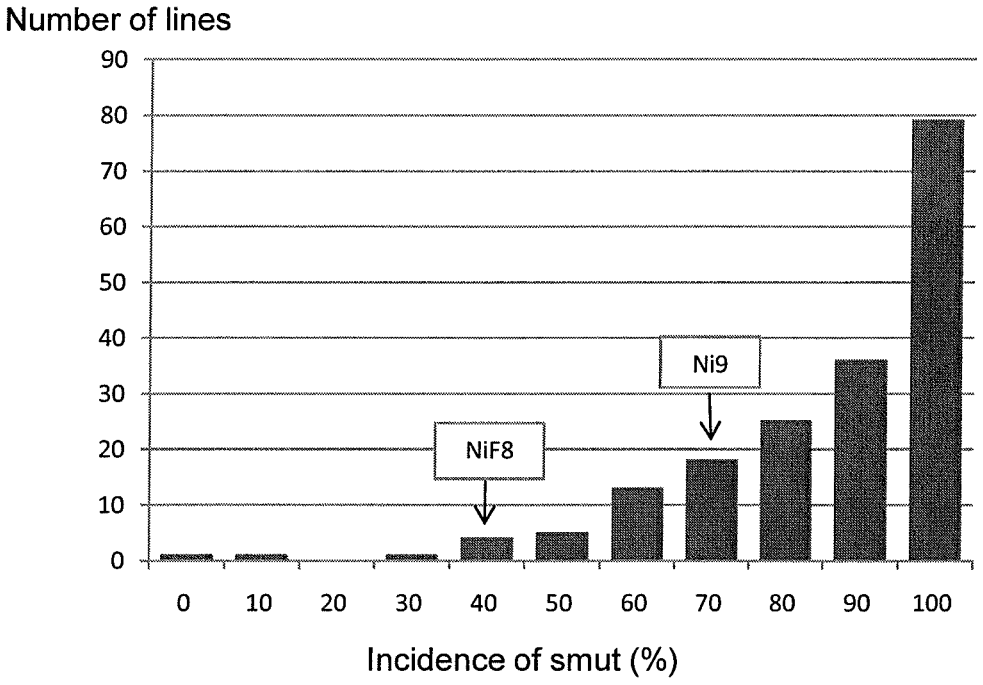
80
90
100
Incidence of smut (%)

Fig. 4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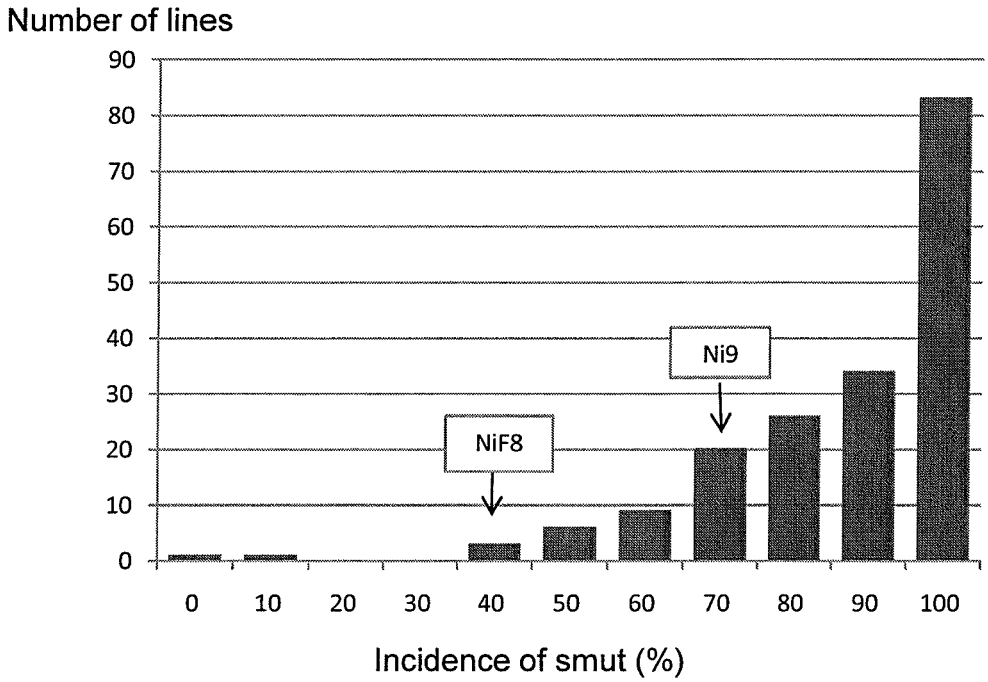
80
90
100
Incidence of smut (%)

Fig. 5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50
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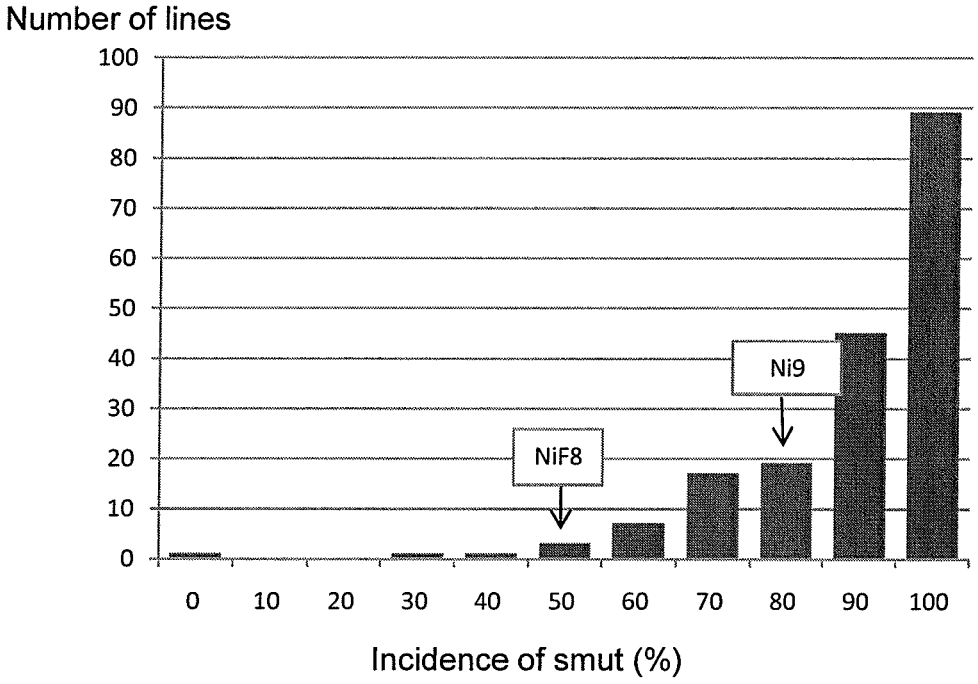
70
80
90
100
Incidence of smut (%)

Fig. 6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100
Incidence of smut (%)

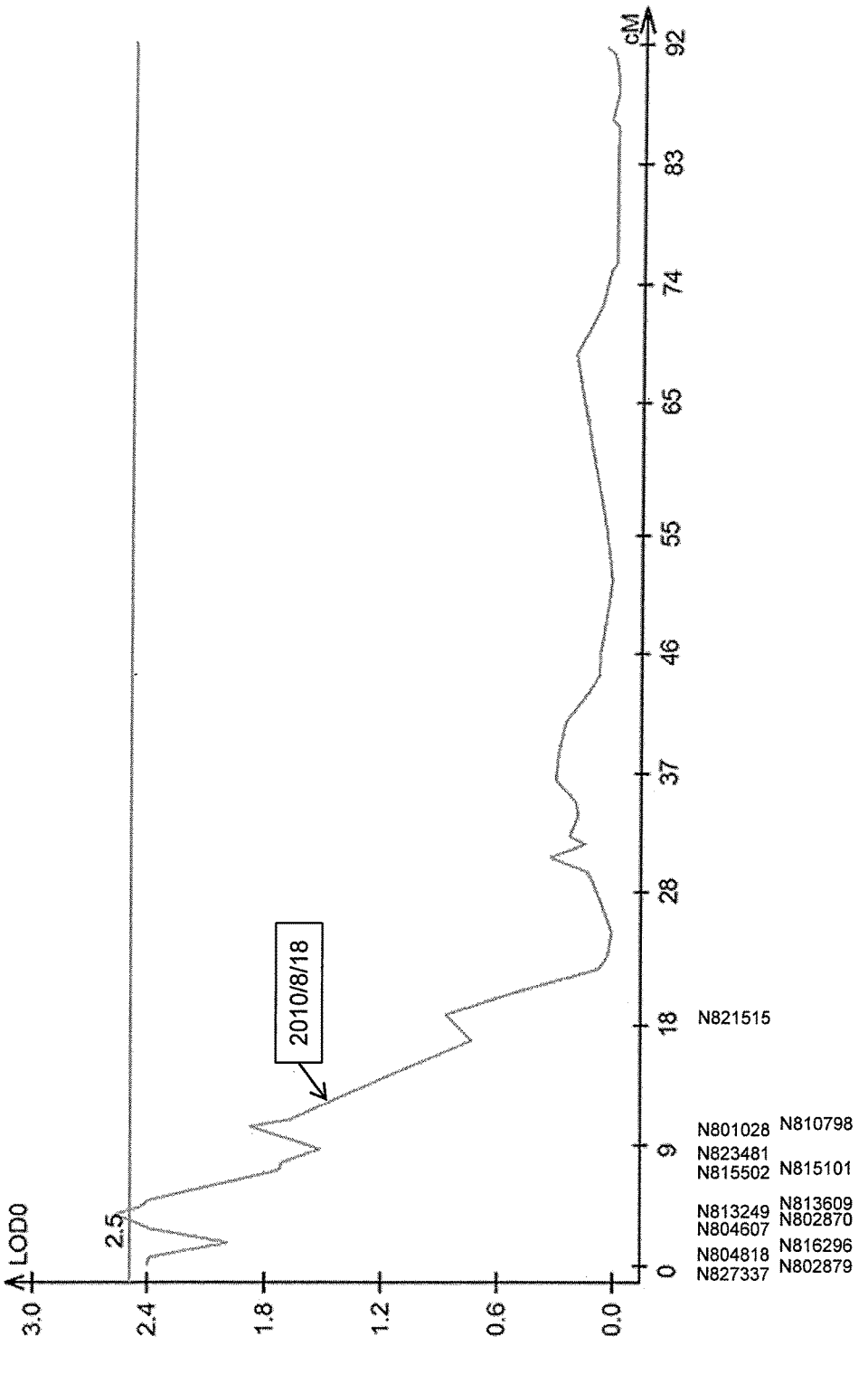
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Fig. 7

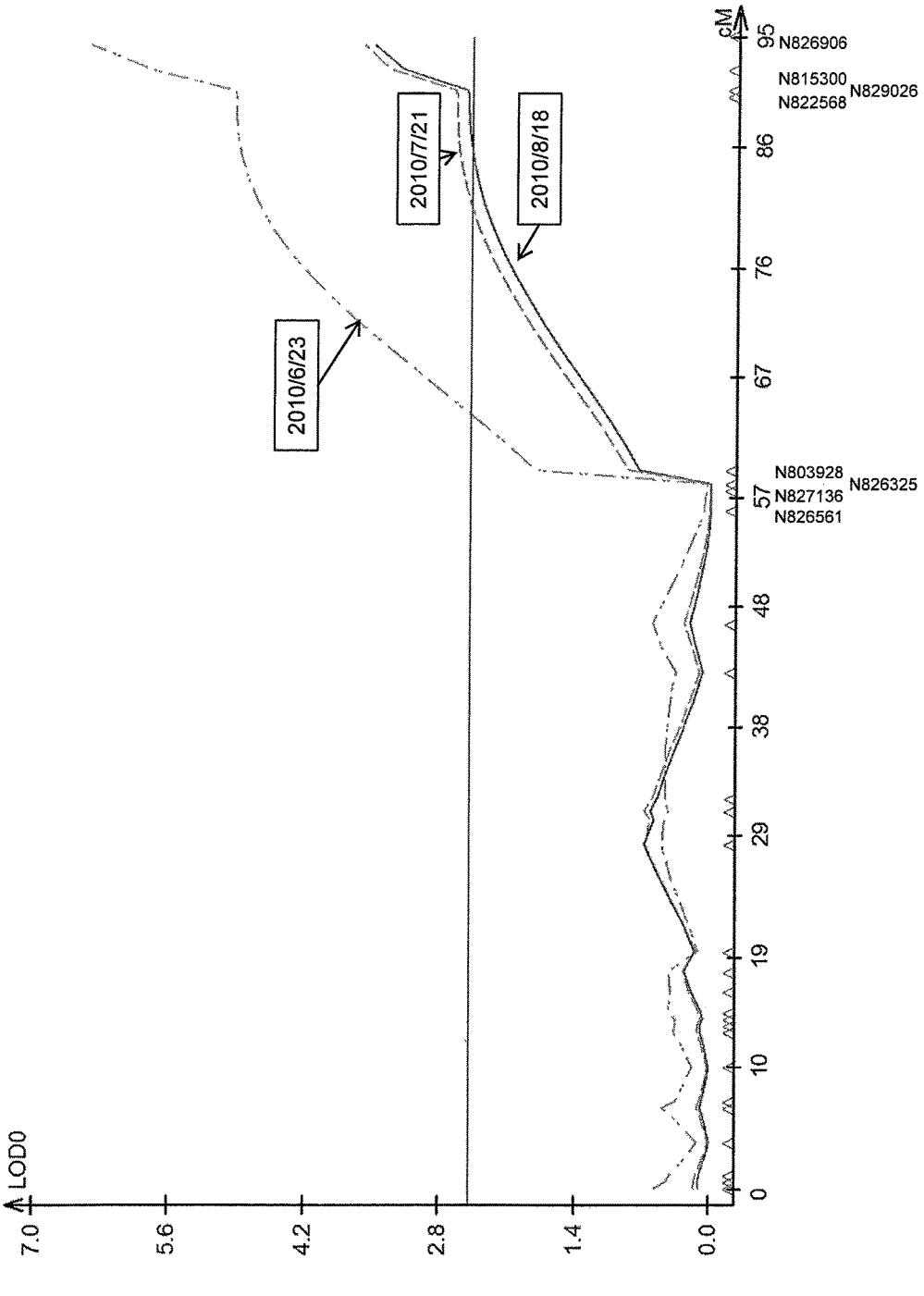
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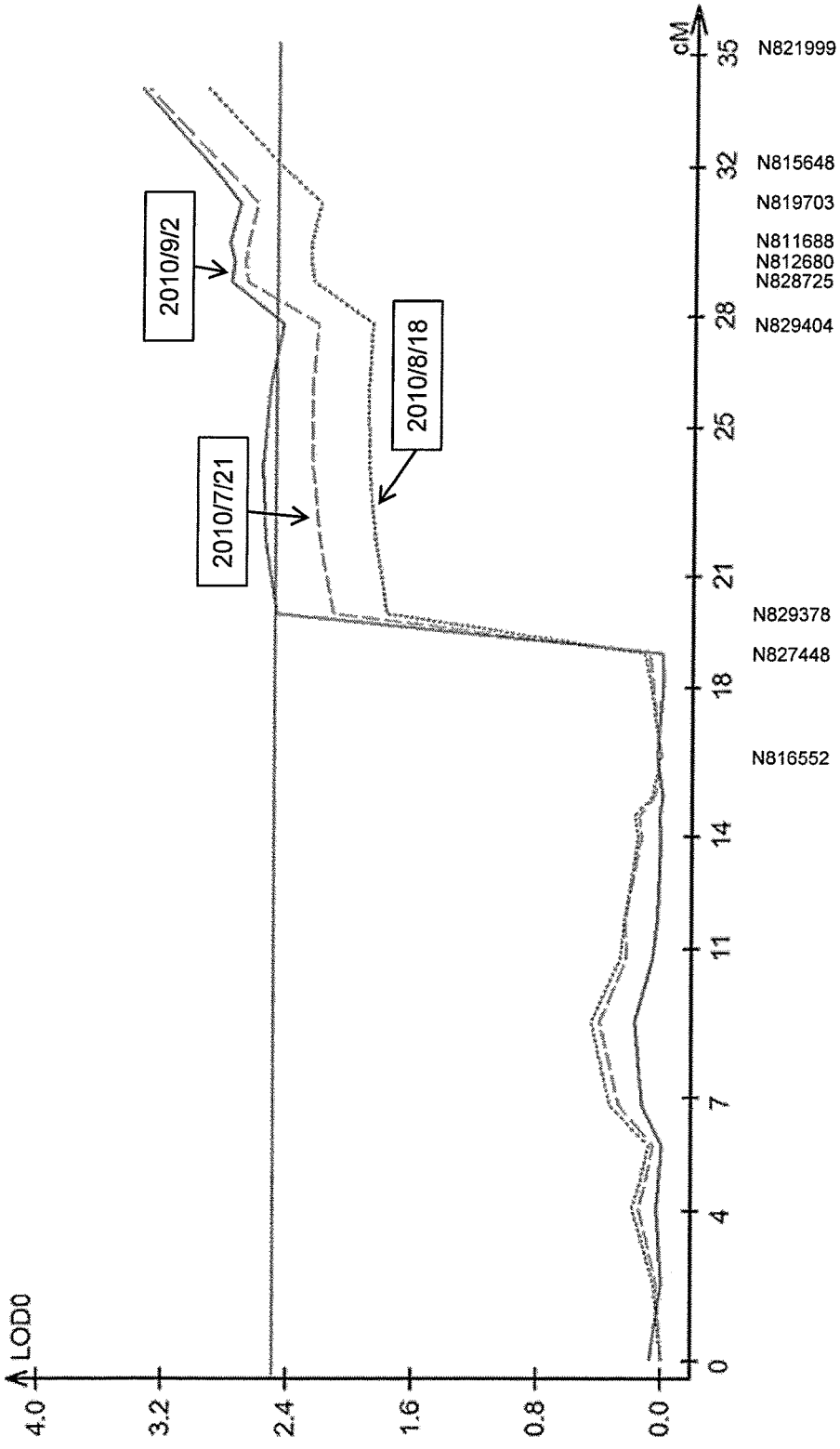
Fig. 8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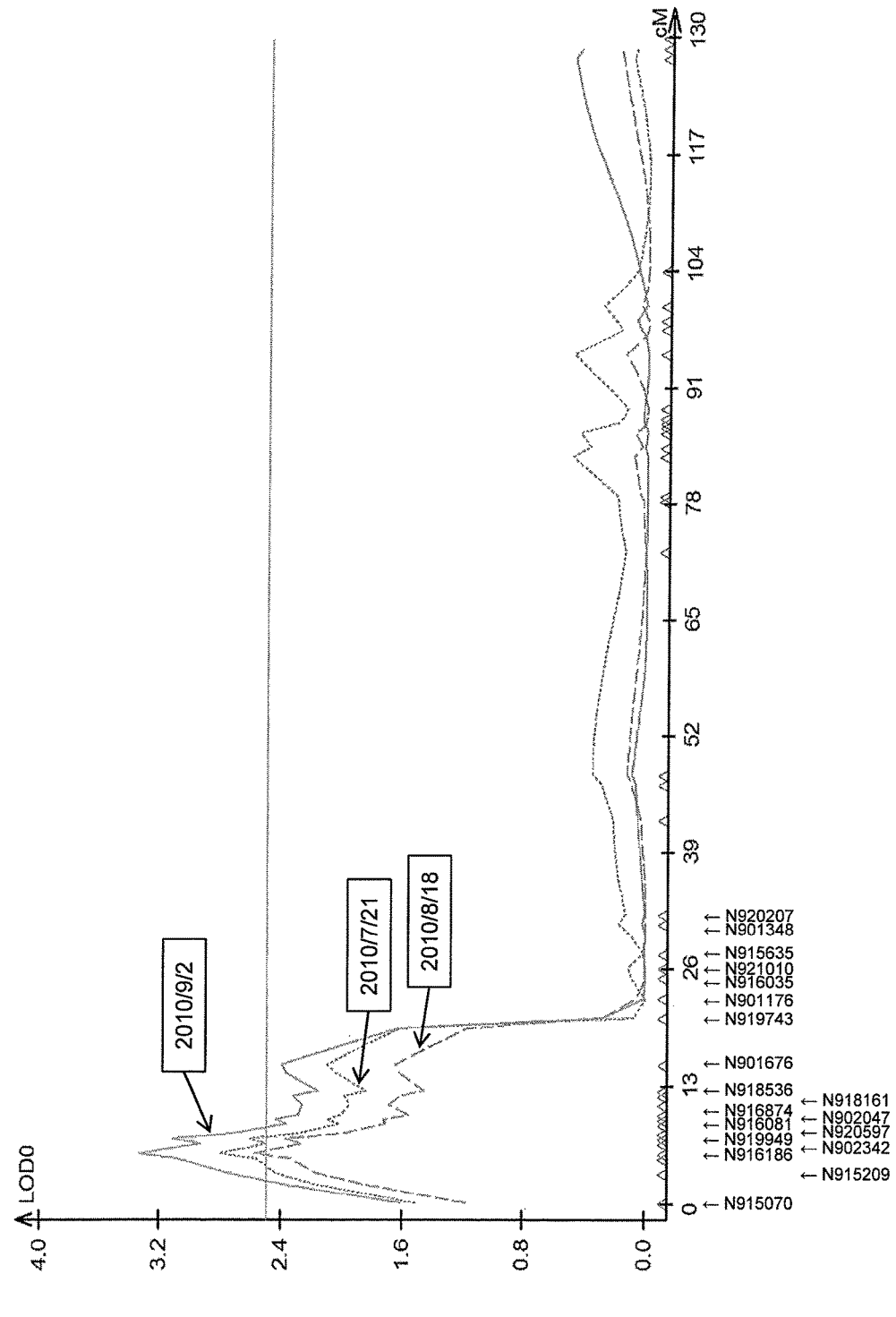
2010/7/21

Fig. 9

Fig. 10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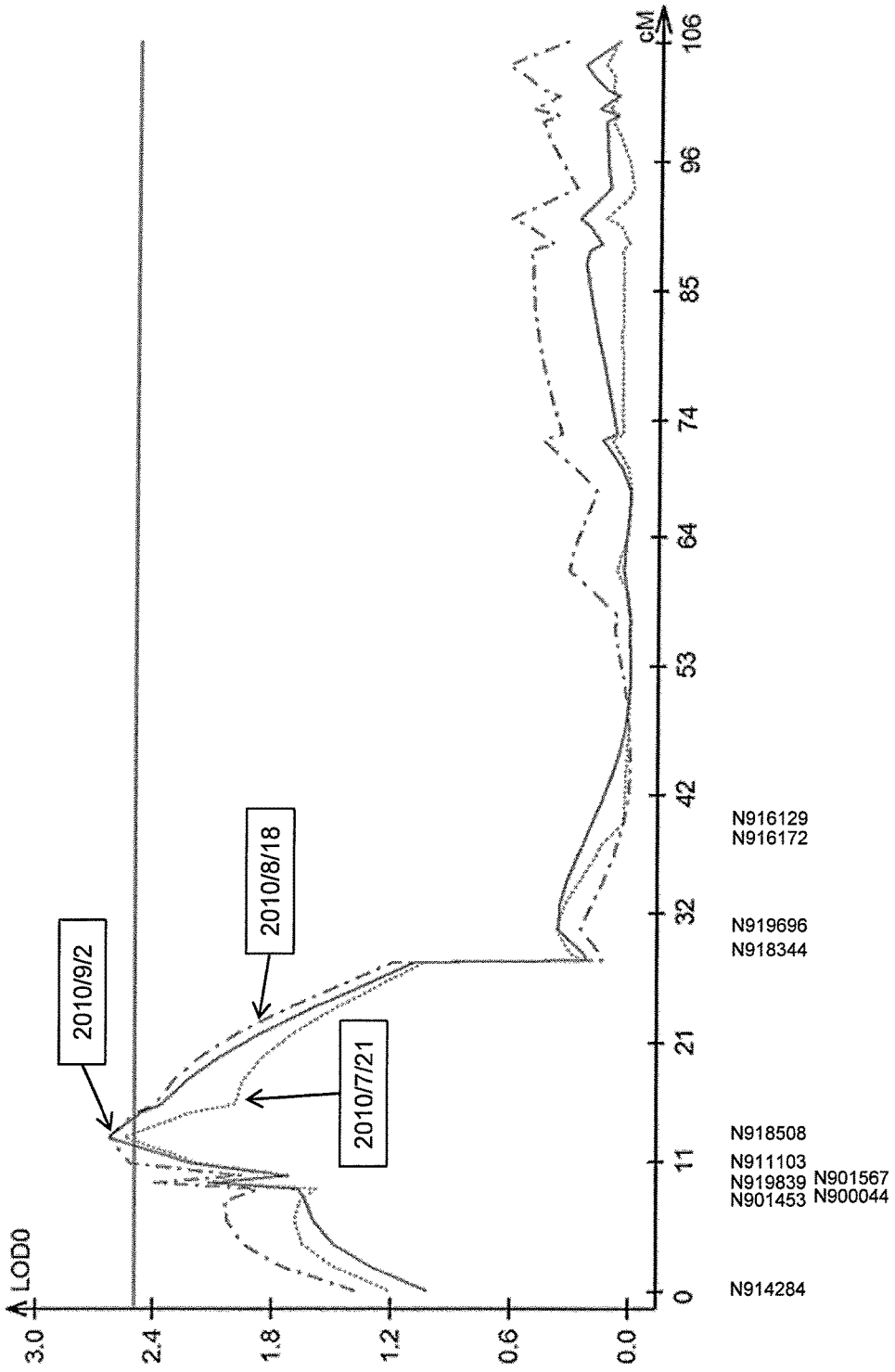
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Fig. 11

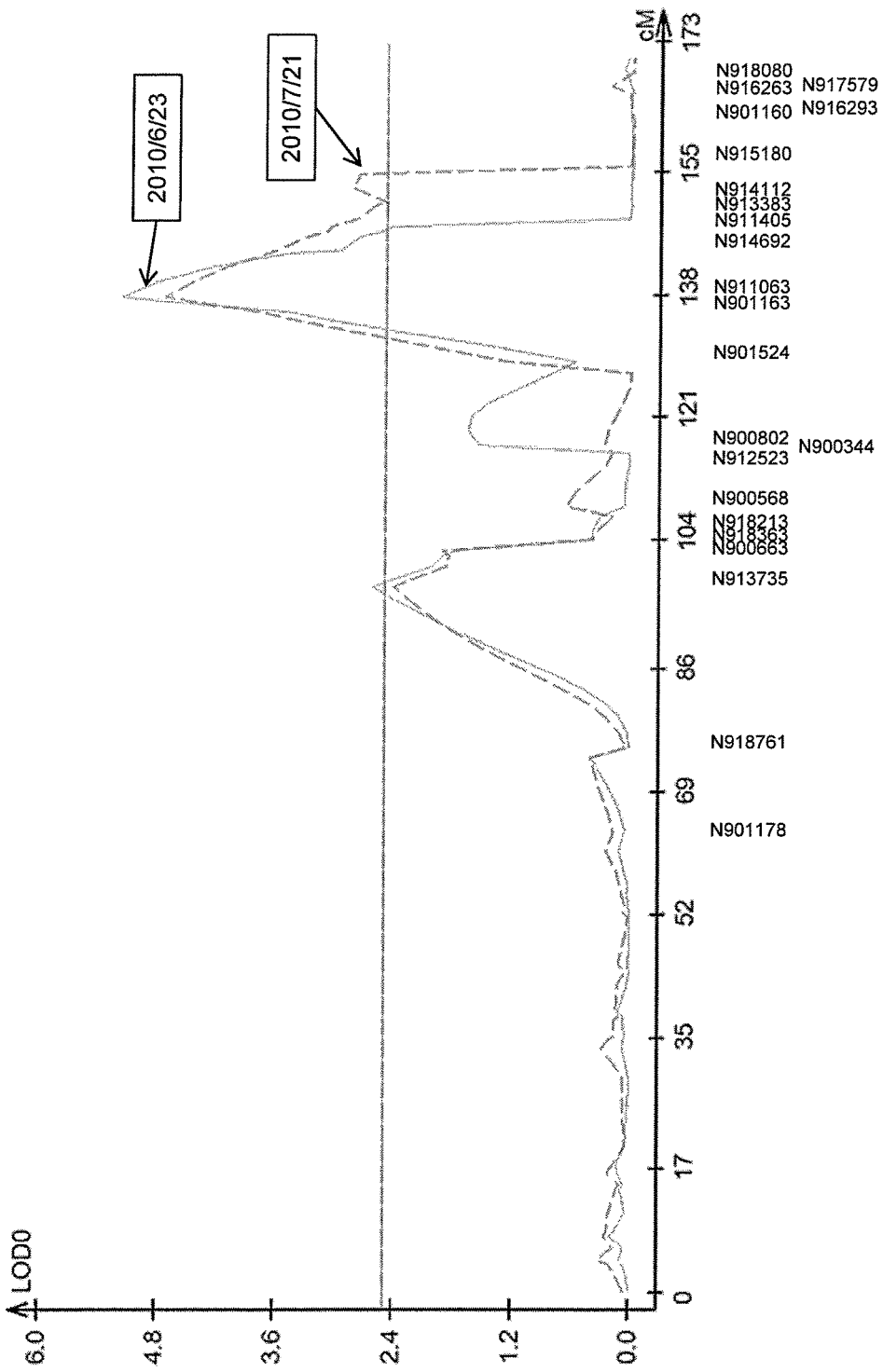
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Fig. 12

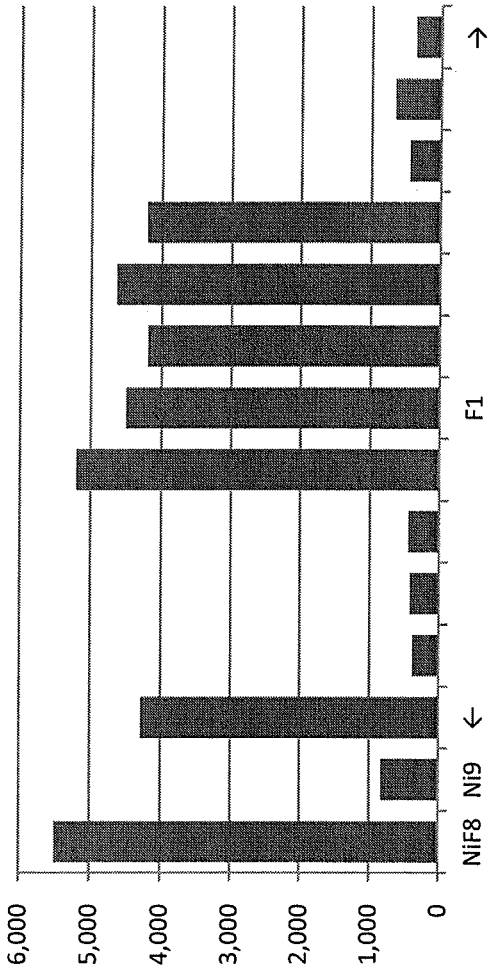


Fig. 13

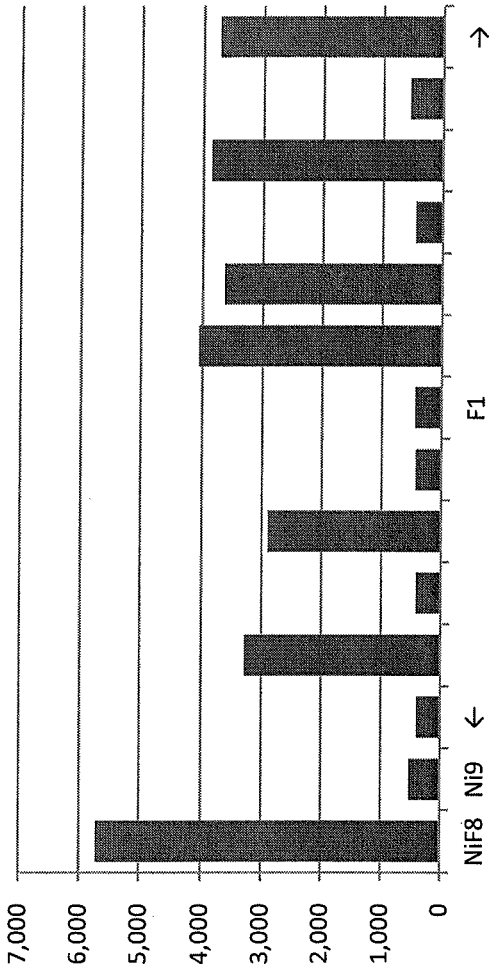
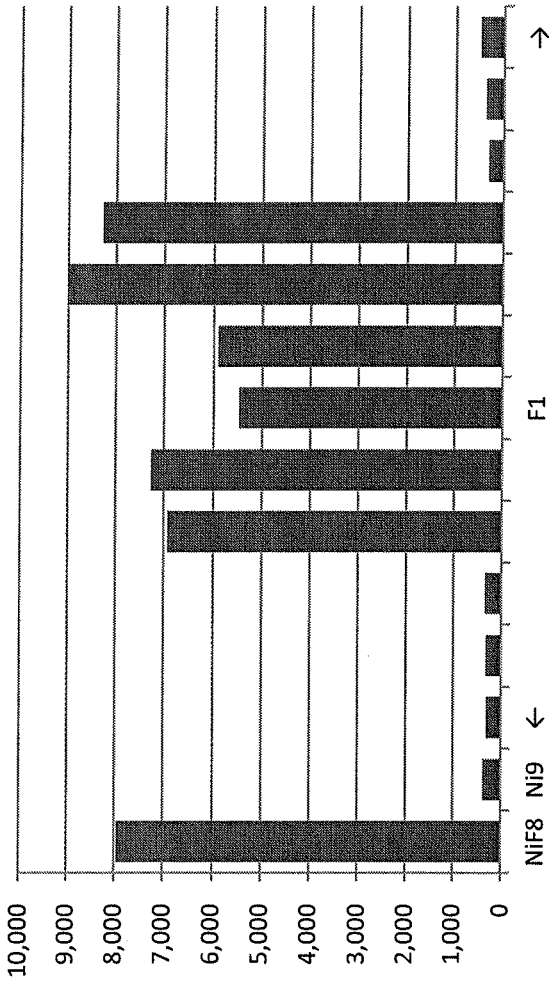


Fig. 14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Ni9
←
F1
→

Fig. 15

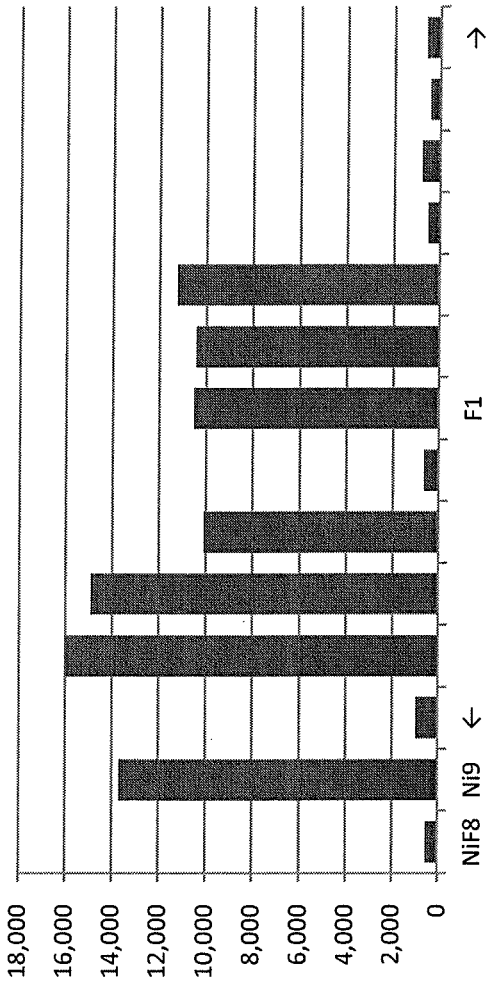
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Fig. 16

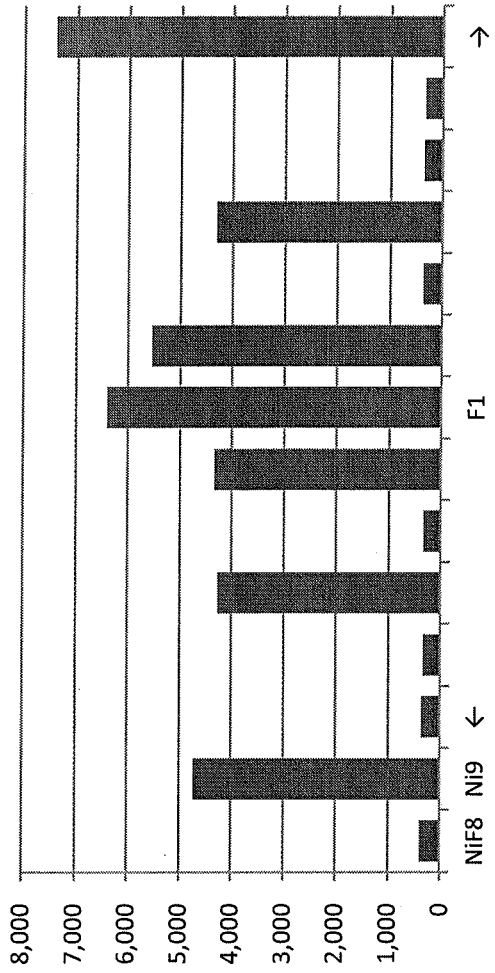

Fig 17

Fig. 18

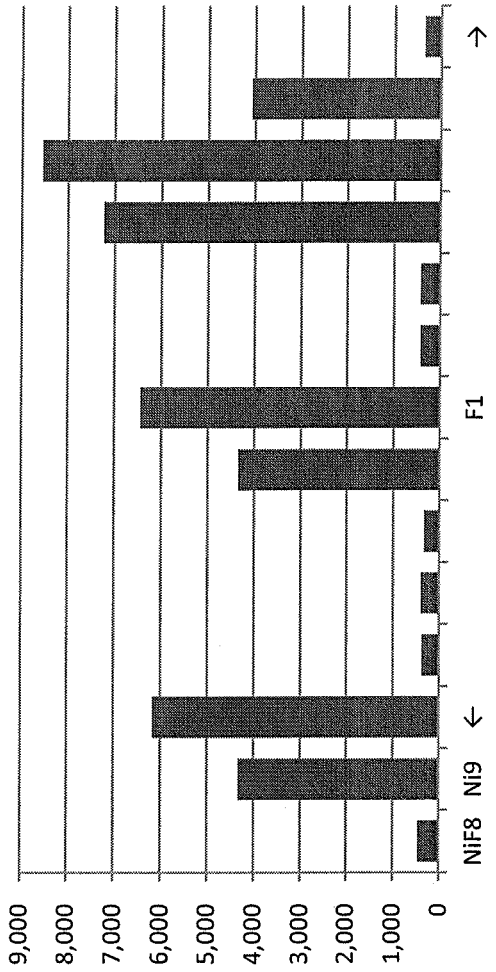

Fig. 19

MARKER ASSOCIATED WITH RESISTANCE TO SMUT IN PLANT BELONGING TO GENUS *SACCHARUM*, AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Divisional of U.S. application Ser. No. 14/113,539 filed Oct. 23, 2013, which is a National Stage of International Application No. PCT/JP2012/060671, filed Apr. 20, 2012, which claims priority to Japanese Patent Application No. 2011-101050, filed Apr. 28, 2011, and to Japanese Patent Application No. 2012-094995, filed Apr. 18, 2012, the contents of all of which are incorporated herein by reference in their entirety.

TECHNICAL FIELD

The present invention relates to a marker associated with resistance to smut whereby a sugarcane line resistant to smut can be selected, and a method for use thereof.

BACKGROUND ART

Sugarcane has been cultivated as a raw material for sugar, liquor, and the like for edible use. In addition, sugarcane has been used as, for example, a raw material for biofuel in a variety of industrial fields. Under such circumstances, there is a need to develop novel sugarcane varieties having desirable characteristics (e.g., sugar content, enhanced vegetative capacity, sprouting capacity, disease resistance, insect resistance, cold resistance, an increase in leaf blade length, an increase in leaf area, and an increase in stalk length).

In general, the following three ways may be used for identification of a plant variety/line: "characteristics comparison" for comparison of characteristics data, "comparison during cultivation" for comparison of plants cultivated under the same conditions, and "DNA assay" for DNA analysis. There are many problems in line identification with characteristics comparison or comparison during cultivation, including reduction of precision due to differences in cultivation conditions, lengthy duration of field research that requires a number of steps, and the like. In particular, since sugarcane plants are much larger than other graminaceous crops such as rice and maize, it has been difficult to conduct line identification based on field research.

In addition, in order to identify a variety resistant to a certain disease, an inoculation test is carried out using a causative microorganism of a disease after long-term cultivation of sugarcane, and then disease resistance data are collected by observing lesions and the like. However, transmission of the causative microorganism to an external environment must be securely prevented when the test is carried out, and thus it is necessary to provide, for example, facilities such as a large-scale special-purpose greenhouse, a special-purpose field or isolation facility from an external environment. Further, for creation of a novel sugarcane variety, first, tens of thousands of hybrids are created via crossing, followed by seedling selection and stepwise selection of desirable excellent lines. Eventually, 2 or 3 types of novel varieties having desired characteristics can be obtained. As described above, for creation of a novel sugarcane variety, it is necessary to cultivate and evaluate an enormous number of lines, and it is also necessary to prepare the above large-scale greenhouse or field and undertake highly time-consuming efforts.

Therefore, it has been required to develop a method for identifying a sugarcane line having disease resistance with the use of markers present in the sugarcane genome. In particular, upon creation of a novel sugarcane variety, if excellent markers could be used to examine a variety of characteristics, the above problems particular to sugarcane would be resolved, and the markers would be able to serve as very effective tools. However, since sugarcane plants have a large number of chromosomes (approximately 100 to 130) due to higher polyploidy, the development of marker technology has been slow. In the case of sugarcane, although the USDA reported genotyping with the use of SSR markers (Non-Patent Literature 1), the precision of genotyping is low because of the small numbers of markers and polymorphisms in each marker. In addition, the above genotyping is available only for American/Australian varieties, and therefore it cannot be used for identification of the major varieties cultivated in Japan, Taiwan, India, and other countries or lines that serve as useful genetic resources.

In addition, Non-Patent Literature 2 suggests the possibility that a sugarcane genetic map can be created by increasing the number of markers, comparing individual markers in terms of a characteristic relationship, and verifying the results. However, in Non-Patent Literature 2, an insufficient number of markers are disclosed and markers linked to desired characteristics have not been found.

Meanwhile, as a marker associated with disease resistance, a marker associated with black root rot resistance in sugar beet disclosed in Patent Literature 1 is known. In addition, a technique of selecting a *Zea mays* variety using a maker linked to a desired trait is disclosed in Patent Literature 2.

The level of infectiousness of the causative microorganism of sugarcane smut is high, and therefore the onset of smut quickly results in the infection of the entire field. Crops of sugarcane affected with smut cannot be used as raw material for sugar production, and even they die. Therefore, the development of smut will cause a significant decline in yield within the following year or later. Damage due to smut has been reported in more than 28 countries, including Brazil, the U.S., Australia, China, and Indonesia. Smut can be prevented by sterilization treatment prior to planting; however, preventive effects are limited to the period of early growth. Thus, cultivation of a sugarcane variety imparted with smut resistance has been awaited.

CITATION LIST

Non-Patent Literature

Non-Patent Literature 1: Maydica 48(2003) 319-329 "Molecular genotyping of sugarcane clones with microsatellite DNA markers"

Non-Patent Literature 2: Nathalie Piperidis et al., Molecular Breeding, 2008, Vol. 21, 233-247

Patent Literature

Patent Literature 1: WO 2007/125958

Patent Literature 2: JP Patent Publication (Kokai) No. 2010-516236 A

SUMMARY OF INVENTION

Technical Problem

In view of the above, an object of the present invention is to provide a marker associated with resistance to smut, which is a quantitative trait of sugarcane.

Solution to Problem

In order to achieve the object, the present inventors conducted intensive studies. The present inventors prepared many sugarcane plant markers and carried out linkage analysis of quantitative traits along with such markers for hybrid progeny lines. Accordingly, the present inventors found markers linked to quantitative traits such as smut resistance. This has led to the completion of the present invention.

The present invention encompasses the following.

(1) A marker associated with resistance to sugarcane smut, which consists of a continuous nucleic acid region existing in a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 1 and the nucleotide sequence shown in SEQ ID NO: 14, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 15 and the nucleotide sequence shown in SEQ ID NO: 22, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 23 and the nucleotide sequence shown in SEQ ID NO: 32, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 33 and the nucleotide sequence shown in SEQ ID NO: 51, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 52 and the nucleotide sequence shown in SEQ ID NO: 62, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 63 and the nucleotide sequence shown in SEQ ID NO: 72, or a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 73 and the nucleotide sequence shown in SEQ ID NO: 85 of a sugarcane chromosome.

(2) The marker associated with resistance to sugarcane smut according to (1), wherein the continuous nucleic acid region comprises any nucleotide sequence selected from the group consisting of the nucleotide sequences shown in SEQ ID NOS: 1 to 85 or a part of the nucleotide sequence.

(3) The marker associated with resistance to sugarcane smut according to (1), wherein the continuous nucleic acid region is located at a position in a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 5 and the nucleotide sequence shown in SEQ ID NO: 9, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 18 and the nucleotide sequence shown in SEQ ID NO: 22, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 25 and the nucleotide sequence shown in SEQ ID NO: 32, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 33 and the nucleotide sequence shown in SEQ ID NO: 42, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 57 and the nucleotide sequence shown in SEQ ID NO: 59, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 64 and the nucleotide sequence shown in SEQ ID NO: 66, or a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 72 and the nucleotide sequence shown in SEQ ID NO: 80 of a sugarcane chromosome.

(4) A method for producing a sugarcane line having improved smut resistance comprising: a step of extracting a chromosome of a progeny plant obtained from parent plants, at least one of which is a sugarcane plant, and/or a chromosome of a parent sugarcane plant; and a step of determining the presence or absence of the marker associated with resistance to sugarcane smut according to any one of (1) to (3) in the obtained chromosome.

(5) The method for producing a sugarcane line according to (4), wherein a DNA chip comprising a probe corresponding to the marker associated with resistance to sugarcane smut is used in the determination step.

(6) The method for producing a sugarcane line according to (4), wherein the progeny plant is in the form of seeds or a young seedling and the chromosome is extracted from the seeds or the young seedling.

This specification includes part or all of the contents as disclosed in the specification and/or drawings of Japanese Patent Application Nos. 2011-101050 and 2012-94995, which are priority documents of the present application.

Advantageous Effects of Invention

According to the present invention, a novel marker associated with resistance to sugarcane smut linked to a sugarcane quantitative trait such as smut resistance can be provided. With the use of the marker associated with resistance to sugarcane smut of the present invention, smut resistance of a line obtained by crossing sugarcane lines can be tested. Thus, a sugarcane line characterized by improved smut resistance can be identified at a very low cost.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** schematically shows the process of production of a DNA microarray used for acquisition of sugarcane chromosome markers.

FIG. **2** schematically shows a step of signal detection with the use of a DNA microarray.

FIG. **3** is a characteristic chart showing data on smut resistance examined on Jun. 23, 2010, for sugarcane variety/line groups used in the Examples.

FIG. **4** is a characteristic chart showing data on smut resistance examined on Jul. 21, 2010, for sugarcane variety/line groups used in the Examples.

FIG. **5** is a characteristic chart showing data on smut resistance examined on Aug. 18, 2010, for sugarcane variety/line groups used in the Examples.

FIG. **6** is a characteristic chart showing data on smut resistance examined on Sep. 2, 2010, for sugarcane variety/line groups used in the Examples.

FIG. **7** is a characteristic chart showing QTL analysis results regarding smut resistance (the 5th linkage group in NiF8).

FIG. **8** is a characteristic chart showing QTL analysis results regarding smut resistance (the 17th linkage group in NiF8).

FIG. **9** is a characteristic chart showing QTL analysis results regarding smut resistance (the 40th linkage group in NiF8).

FIG. **10** is a characteristic chart showing QTL analysis results regarding smut resistance (the 1st linkage group in Ni9).

FIG. **11** is a characteristic chart showing QTL analysis results regarding smut resistance (the 13th linkage group in Ni9).

FIG. **12** is a characteristic chart showing QTL analysis results regarding smut resistance (the 14th linkage group in Ni9).

FIG. **13** is a characteristic chart showing signal levels of N802870 for individual lines.

FIG. **14** is a characteristic chart showing signal levels of N827136 for individual lines.

FIG. **15** is a characteristic chart showing signal levels of N812680 for individual lines.

FIG. **16** is a characteristic chart showing signal levels of N916081 for individual lines.

FIG. **17** is a characteristic chart showing signal levels of N919839 for individual lines.

FIG. **18** is a characteristic chart showing signal levels of N918761 for individual lines.

FIG. **19** is a characteristic chart showing signal levels of N901160 for individual lines.

DESCRIPTION OF EMBODIMENTS

The marker associated with resistance to sugarcane smut and the method for using the same according to the present invention are described below. In particular, a method for producing a sugarcane line using a marker associated with resistance to sugarcane smut is described.

<Markers Associated with Resistance to Sugarcane Smut>

The marker associated with resistance to sugarcane smut of the present invention corresponds to a specific region present on a sugarcane chromosome and is linked to a causative gene (or a group of causative genes) for a trait characterized by smut resistance. Thus, it can be used to identify a trait characterized by smut resistance. Specifically, it is possible to determine that a progeny line obtained using a known sugarcane line is a line having a trait characterized by the improvement of smut resistance by confirming the presence or absence of the marker associated with resistance to sugarcane smut in such progeny line. In the present invention, the term "smut" refers to a disease characterized by lesion formation due to infection with a microorganism of the genus *Ustilago*. One example of a microorganism of the genus *Ustilago* is *Ustilago scitaminea*.

In addition, the term "marker associated with resistance to sugarcane smut" refers to both a marker linked to a trait characterized by the improvement of smut resistance and a marker linked to a trait characterized by the reduction of smut resistance. For example, if the presence of the former marker in a certain sugarcane variety is confirmed, it is possible to determine that the variety has improved smut resistance. Further, if the presence of the former marker and the absence of the latter marker in a certain sugarcane variety are confirmed, it is possible to determine that the variety has improved smut resistance with high accuracy. It is also possible to determine that a certain sugarcane variety has improved smut resistance by confirming only the absence of the latter marker.

The term "sugarcane" used herein refers to a plant belonging to the genus *Saccharum* of the family Poaceae. In addition, the term "sugarcane" includes so-called noble cane (scientific name: *Saccharum officinarum*) and wild cane (scientific name: *Saccharum spontaneum*), *Saccharum barberi, Saccharum sinense*, and the earlier species of *Saccharum officinarum* (*Saccharum robustum*). The term "known sugarcane variety/line" is not particularly limited. It includes any variety/line available in Japan and any variety/line available outside Japan. Examples of sugarcane varieties cultivated in Japan include, but are not limited to, Ni1, NiN2, NiF3, NiF4, NiF5, Nib, NiN7, NiF8, Ni9, NiTn10, Ni11, Ni12, Ni14, Ni15, Ni16, Ni17, NiTn19, NiTn20, Ni22, and Ni23. Examples of main sugarcane varieties used in Japan described herein include, but are not limited to, NiF8, Ni9, NiTn10, and Ni15. In addition, examples of main sugarcane varieties that have been introduced into Japan include, but are not limited to, F177, Nco310, and F172.

In addition, a progeny line may be a line obtained by crossing a mother plant and a father plant of the same species, each of which is a sugarcane variety/line, or it may be a hybrid line obtained from parent plants when one thereof is a sugarcane variety/line and the other is a closely related variety/line (*Erianthus arundinaceus*). In addition, a progeny line may be obtained by so-called backcrossing.

The marker associated with resistance to sugarcane smut of the present invention has been newly identified by QTL (Quantitative Trait Loci) analysis using a genetic linkage map containing 3004 markers and 4569 markers originally obtained from sugarcane chromosomes, and sugarcane smut resistance data. In addition, many genes are presumably associated with sugarcane smut resistance, which is a quantitative trait characterized by a continuous distribution of sugarcane smut resistance. That is, sugarcane smut resistance is evaluated based on the incidence of smut characterized by such continuous distribution. For QTL analysis, the QTL Cartographer gene analysis software (Wang S., C. J. Basten, and Z.-B. Zeng (2010); Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, N.C.) is used, and the analysis is carried out by the composite interval mapping (CIM) method.

Specifically, seven relevant regions included in the above genetic linkage map with LOD scores equivalent to or exceeding a given threshold (e.g., 2.5) have been found by QTL analysis described above. That is, the following 7 regions have been specified: an approximately 18.7-cM (centimorgan) region including the relevant region, an approximately 39.2-cM region including the relevant region, an approximately 19.2-cM region including the relevant region, an approximately 32.0-cM region including the relevant region, an approximately 39.5-cM region including the relevant region, an approximately 53.4-cM region including the relevant region, and an approximately 38.0-cM region including the relevant region. The term "morgan (M)" used herein refers to a unit representing the relative distance between genes on a chromosome, and it is expressed by the percentage of the crossover rate. In a case of a sugarcane chromosome, 1 cM corresponds to approximately 2000 kb. In addition, it is suggested that a causative gene (or a group of causative genes) for a trait that causes the improvement of smut resistance could be present at the peak positions or in the vicinity thereof.

The 18.7-cM region is a region that comprises 14 types of markers listed in table 1 below in the order shown in table 1 and is linked to a trait characterized by the reduction of smut resistance.

TABLE 1

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
NiF8_5	N827337	CCTCGTCATGC ACCCGTGCCTC TTCTTCCTCTT GCTGTTGCTCC TCCTCC	1,000	SEQ ID NO: 1
	N802879	GGAATTGTTGT AGATTTGTTTT GTGATGGAAAG ATCATACCTCA GCTACAAGAAG TAAATATCCTT TTCCA	1,000	SEQ ID NO: 2

TABLE 1-continued

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
	N804818	GGCATTAGAAG AAAGGTGGAAG AATAAGGTTTG AGCCCTTATTT ATTTGCTTTGG TGATGGAT	1,000	SEQ ID NO: 3
	N816296	CCATTCTACTT CTACCAACCAT AAAACAGGAGG AGCATGCATGC ACATGC	1,000	SEQ ID NO: 4
	N804607	ATTGCTTGCTC GCTGCAACTTG GGCCATGTTTA GTTCCTCGAAT TTGAGT	1,000	SEQ ID NO: 5
	N802870	AGTGAAGAGAT TGGATTTCTAG GGTTACTTTAT AAAGTGTCAAC ACCTTAGATCT GTTTTTTAGT	1,500	SEQ ID NO: 6
	N813249	GGCCGGCACGA GCATCAGGGTC AAGACTCAAGA GCTCAAGTGCT TGCTTT	1,500	SEQ ID NO: 7
	N813609	TACTTTGTCTC GTTCCAGTAGT CCATCAAGCAA GCCTCGTACAC AAGTCC	1,000	SEQ ID NO: 8
	N815502	TGCACTGGGGA TACCAGTTGAG TTGATTGCACA ACTTGCGCTAC ACCATG	1,000	SEQ ID NO: 9
	N815101	GCCGCCTGATG GAAACGGTCGT CGCATCCAAAG ACGCACATGGT TTAGCA	1,000	SEQ ID NO: 10
	N823481	AGTACCTGTTC TGCTGCACTAC ATAACAGTACT TTTCAGTGAAC GAACAGTGTTT TC	1,000	SEQ ID NO: 11
	N801028	AGCGGATAGCG CTAGCATGTCA TTCTCTCCCCT CGCTAGCACGT TATTCC	1,500	SEQ ID NO: 12
	N810798	GTTGCGGCGTG TGTTGATGATG TAAAGAATACT CGTCCGTGAGA AATTATCA	1,500	SEQ ID NO: 13
	N821515	ACGTGACGACG ACGACGATGCA GCTGGGGCTTG GCGTGGAATGG TTGTCG	1,000	SEQ ID NO: 14

The 39.2-cM region is a region that comprises 8 types of markers listed in table 2 below in the order shown in table 2 and is linked to a trait characterized by the improvement of smut resistance.

TABLE 2

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
NiF8_17	N826561	GGCCTTGTTTA AATGTCACCTA AATTCTAAATT TTACACTCTTT TCATAACATCG AATCTTAAAA	1,000	SEQ ID NO: 15
	N827136	AAACTGAGGGA TTACTTTCCAA TTGAAATGTCA TCCACCACAAA CACAAAAGGCA TACTCA	1,500	SEQ ID NO: 16
	N826325	ACACTACACTG TGTAGGCAATG AGCAGCTCTGT TGCACAGCAAA GCCAAA	1,000	SEQ ID NO: 17
	N803928	GGATGTGAAGT ATGTATGTGTT TTCAGATGGAC CAAGGAAGCTG CATGGG	1,000	SEQ ID NO: 18
	N822568	TACGGTGGTAC AAAGCTTAGAT CAATGATCAAG CTACAAAACAC ACAAAGATAGT CAGTAGAAAAA GT	1,000	SEQ ID NO: 19
	N829026	GACGACGAGGT GGGCAGCGCCA GTGCGCTACTA CCTTCTTTCTT GCAACT	1,000	SEQ ID NO: 20
	N815300	GTATGGTTATG TTGGTACTAAA GGTTTCTGACT ATTGTATTGTA TTGTTGTGTTA TAATGGGTTCA ATG	1,000	SEQ ID NO: 21
	N826906	GGCTGCAATAC CTGTTCCTCAT CTCATCTATTC GTGCAAAGTTG CTGGTC	1,000	SEQ ID NO: 22

The 19.2-cM region is a region that comprises 10 types of markers listed in table 3 below in the order shown in table 3 and is linked to a trait characterized by the reduction of smut resistance.

TABLE 3

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
NiF8_40	N816552	TCGGGTTGGAG GCAAGGAAGAA AGGAGCTAGAT TGCTCGGCTGC TGGTGC	1,500	SEQ ID NO: 23
	N827448	ACAGTAGTGCA ACTGCGACGAC GATGTGTGGGT ATATGTTCCAT AGCTTG	1,000	SEQ ID NO: 24
	N829378	TTTTGATTGGC CTTGCAGATGT TGCAGCGATGG CACTCGTGGCA AACAGA	900	SEQ ID NO: 25

TABLE 3-continued

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
	N829404	AACCATGCTGA AAACGTCTTCC GTTTACAGTTT ATGGTATATCC GCTTAAAACTA ACTCGATC	1,000	SEQ ID NO: 26
	N828725	AATCTAAATGA CTAATGAGACC GTGAGAGCTGC TTAGCTTAATG GTGCATCCCTT TTTAAACT	1,000	SEQ ID NO: 27
	N812680	AAGAACACTGC TAAGGATGGTC ACAATTTGGAA ACTGAAGTTTT ATCTCTGGTTC GGT	1,500	SEQ ID NO: 28
	N811688	AAGCTGCATCT GATTCTCATCC AAACCTGCTCT GCTCATTATCA TTACTTCGT	1,000	SEQ ID NO: 29
	N819703	CCAACCAACAG CAAGAACACCA AGACGCACATA ATGAGGCCCAT GAAGTA	1,500	SEQ ID NO: 30
	N815648	TTTACACCAGT GAACTGACAAA AAATCGAAGTG GTGCGGTACAT AAGAACATTTA CATCCAACT	1,000	SEQ ID NO: 31
	N821999	GACCAATCTAG GAAAAACAATT GCACAAATGAC TACATTTATTA TGGCAAATCAA TTTTCTTCAGT CATTGTA	1,000	SEQ ID NO: 32

The 32.0-cM region is a region that comprises 19 types of markers listed in table 4 below in the order shown in table 4 and is linked to a trait characterized by the improvement of smut resistance.

TABLE 4

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
Ni9_1	N915070	ATAGTCTACCT ATACTGGTGCC ACAAGTCAACA AGTGATGGCAA TACCCATTCAA ATT	1,000	SEQ ID NO: 33
	N915209	TGGCAATACCC ATTCAAATTGC GTCAAATGTGA ATAAATGGAGG TAGATGACTAA CACCTTTGTTT CAAAA	1,000	SEQ ID NO: 34
	N916186	CTGCAATACAA TGCGGTGGAAG CGGATTGGTGG AAGGCATGCAT GCATCA	1,000	SEQ ID NO: 35
	N902342	CCAAATACCTA AGTGCACTTTT TTCTGAGGCCA	900	SEQ ID NO: 36
		AATACCTAGGT TCGAAAGATTC GT		
	N919949	CCGCCTCAAAA GGAAGTAACAC AGGAACATGAT CATACGGAGTA GTACTAT	1,000	SEQ ID NO: 37
	N920597	CTTGCCGGCCG GGACCCTGCTG GCACGATCAAG CGACTACAGTA CAATGC	1,500	SEQ ID NO: 38
	N916081	CAAAGAAAGCA CATTACCGCGT ATGTTACCAAC TTCCTATGTTG ACTATCCAAAT ACTG	4,000	SEQ ID NO: 39
	N902047	GGATTGGTCTA GTACAATCTTT ATTGAAGACGA AAGATTTATGC ATGGTGATTAG TTGAGCCTGT	1,500	SEQ ID NO: 40
	N916874	CAAATATGACG ATGGAAATATA TAGTACTATTA ATAAGACATAA CTTGCAGCATA TATTAATTTCA TAGGATAAG	1,000	SEQ ID NO: 41
	N918161	CTAGTTAGAGC ATCTCCAAGCG TACTCAGAAGA GTCGCCCAATC TAGCAA	1,000	SEQ ID NO: 42
	N918536	CAGAGAAACTG GGAACGAAACA GGACAATACAT CTGTACGTTTG GCTTGT	900	SEQ ID NO: 43
	N901676	TCCCTGTACTG TATGGTCGCCA CAAATGCATAT TGATAGACATG TTTATGATGTA GAATTTGATGT TTACA	1,000	SEQ ID NO: 44
	N919743	AAATCAATAAA GAAAGGCACGC TGAAAATAAGA TGGTCTGATCG AGCTCCTGTGT TTAGTACAA	1,000	SEQ ID NO: 45
	N901176	ATTCCAATGAA CTAAGGGTAAG TAGAGATTATT ATATATAAATC AATGATACACA AACTGATCAAT CAACTAA	1,500	SEQ ID NO: 46
	N916035	GCCTTCTTGAT CTCTCAGACTA AGAACATAGGC CCAGAGTGAGG GGAAAC	1,500	SEQ ID NO: 47
	N921010	CGTTCGCTTGA GCTTATTAGAT AAAATCAATCA GCAATAAAATA ATATTTTTTTC TAATAAAAATC AGCA	1,500	SEQ ID NO: 48
	N915635	TTTATCAGCTT CGGAAATCAGC	4,000	SEQ ID NO: 49

TABLE 4-continued

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
		TTGAGCTGACG AAGACATCAAT CTTCTACATCA GAT		
	N901348	ACATGTATGTG CAAAATATCTT GAGACCCTCTG CTTTAACATGC ATGTCCTTCAC ATGT	1,500	SEQ ID NO: 50
	N920207	CAGCTCTGTCA TTGCCGCCAAA CACATATGCGC CTTCATGCCCT TCTCCC	1,500	SEQ ID NO: 51

The 39.5-cM region is a region that comprises 11 types of markers listed in table 5 below in the order shown in table 5 and is linked to a trait characterized by the reduction of smut resistance.

TABLE 5

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
Ni9_13	N914284	AGCCATCCCGC AGAGGCTCTTG ATGTCCTTTGA GCTGTCCTAAA ACCACT	1,000	SEQ ID NO: 52
	N901453	CTATGTGTTGG GCTTATATGTG ATGCATCTTTC CTTTTGAATTC AGGGTAGTGCT GATA	1,000	SEQ ID NO: 53
	N900044	GTGCTGATACG CCACCAGCCGA AACAAATGGTG ATAGCTCTAGC GCACAG	1,000	SEQ ID NO: 54
	N919839	AAATCCTGAAG GCCGAAGCCCG TAGACATGTTC ACCCTAGCAAA CAAAGG	1,500	SEQ ID NO: 55
	N901567	GCATCGGCTGG TGCTGGTAGGG ATAAACCTCTG CTCCGCTTGAT ATTTTT	1,500	SEQ ID NO: 56
	N911103	TTCGCTTGAGT TTTATCAGCAG AATTAACAGTT ATATAGCGGTG TTTTTTCTCTC ACACTAAATCA GTAAA	800	SEQ ID NO: 57
	N918508	CTTGCCTACTT CTTGCATAGAT GCTTAGTTTAC ATTTTACCTGA AATTTATTAAT ATCGATCACTA CAAAT	1,500	SEQ ID NO: 58
	N918344	GAACAAGGAGC ATCCATATATG TATGGCACTTT GACATTGTTGG CTATGTCTAGC TT	1,000	SEQ ID NO: 59

TABLE 5-continued

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
	N919696	GGAAAAGCAAG CAGCTCGTGTA GCAATAGTTGG CATTGGCAACA GACGCC	1,000	SEQ ID NO: 60
	N916172	GGTAAAATTAT GCAAGTTCCCA CGAAATTTGGC ATATGAAAGTG CCCTTAAAAAT TAAGGTTT	1,500	SEQ ID NO: 61
	N916129	GAGCTTTTATT TATGCTAACCT GTAACAATAAA TTGTCTTTGAG CATGGTTTGTT TGATGATCTCA ATGACCG	1,500	SEQ ID NO: 62

The 53.4-cM region is a region that comprises 10 types of markers listed in table 6 below in the order shown in table 6 and is linked to a trait characterized by the reduction of smut resistance.

TABLE 6

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
Ni9_14_1	N901178	ATCTACACAAC AAATCCACTGT ATTAGACGATT GTTATCAAATG ATCTTCCAGCA AATTGACATAA TATGACATT	1,000	SEQ ID NO: 63
	N918761	AGAACAGGGCC ATCGTTGTTAG CGTGCGTGCTG TAAGTTTGATT TAATTTAAAAA AAATACGTATA	1,500	SEQ ID NO: 64
	N913735	ACGTACAAATG TTTGGGATGGC AGAGGACATGT AGTACAGGGTT GATTCTTTTCA ATA	1,000	SEQ ID NO: 65
	N900663	GCACCTCGCTC CTCCTTATCAA GTTTCGATTTC TGGATTTGCTG CTCTTG	1,000	SEQ ID NO: 66
	N918363	AAGGCGAACAA ATGATTCCCCT CAGTGACCTGA ACGTAATAGTA AAATGATACAC ACT	1,000	SEQ ID NO: 67
	N918213	TCGCATGTCAG GGCTGACAAAT GGCTAAAACCA GACGGAAGATA GACGGA	1,500	SEQ ID NO: 68
	N900568	AACATCAGCTT AGTCTTTAGAG GTTATACCTGC TGTGCTATTTT TTTTACTTAGT GTACACCATTC CTGA	1,000	SEQ ID NO: 69
	N912523	CCTTAATCACG CTTGTGAAATA	1,500	SEQ ID NO: 70

TABLE 6-continued

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
		TCACTCAAACC AACAATATCAA TACCACCATTA ATTATGCTTGT GAAATATGC		
	N900344	TTAAAGACTGA AAGAAACAATT ATTGAATTAAA GAACAACTAGA TAGAGAGCACT GGACTGAATGG TTGCAGA	1,500	SEQ ID NO: 71
	N900802	ATCCCATCACA AAGGAAAGAAT TGCACAAACAA TGACGTGGTAC CTTTAAAAGAT AGAGAATGGAA TAGA	1,500	SEQ ID NO: 72

The 38.0-cM region is a region that comprises 13 types of markers listed in table 7 below in the order shown in table 7 and is linked to a trait characterized by the improvement of smut resistance.

TABLE 7

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
Ni9_14_2	N901524	AAGCAACAGAT GACTAGAAGTA CAGTGCAGGAG ACTCCAACACT TTACTATATTA GTAGAAGA	1,000	SEQ ID NO: 73
	N901163	TCTTCAGTTCA TATCTATCATC TATCCGTCGCT CGTTTCATGAG ACAGATCAAAT AAGCAGAT	1,000	SEQ ID NO: 74
	N911063	TTCGAGAATGA GCGCATTAGCA CAAGGTTTAAT TTCATTAATCA CTTTAGGTATC TAGTTAGGTGT GTGT	1,000	SEQ ID NO: 75
	N914692	CGCCCACCAAT GCATTACCCAA TGGGGTACCCG ATGCCGCCCCA TTCGCA	1,500	SEQ ID NO: 76
	N911405	GTGCAGGGTAC CCGTCAATGGG CTACGGCTATG GCCGCCCACCA ATGCAT	1,000	SEQ ID NO: 77
	N913383	AAGATAAATTT ACAAGCAAAAT TAGAATGTCAA ATACCACAAAT ATTGAGAGCTG TGCCTGACAAT TGAGGAGA	1,000	SEQ ID NO: 78
	N914112	AGCTGTGCCTG ACAATTGAGAG TGAACAGAGTA CATTTCATACT GCCCAG	1,000	SEQ ID NO: 79
	N915180	TCCGGAGATTA CAACGTCTTCA	1,000	SEQ ID NO: 80

TABLE 7-continued

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO:
		GTGACGAGAAC CCGAACAGCTG CTCGGT		
	N901160	CCCCTGACACG ATATTTATTTG CCAGAATTTAT GAATTACAGCC GCATTTCGTTG TGT	1,500	SEQ ID NO: 81
	N916293	TTGGCAATCAT CGACTAATTAG GTGTAAAAGAT TCGTCTTGTTA TTTTCTACCAA ATTATGAAATT TA	1,000	SEQ ID NO: 82
	N916263	TATAGGGCCAG ATAAACCATGA TAATCATAGGA TATTTGCAGAA ATCTTAAATTT CTGAGATTGCC AACAGAAGA	1,000	SEQ ID NO: 83
	N917579	TATGGATCTTC CAGTTGATTAC TGTTCTTTCGC TCCGCTTTTTG CTTTTTTACTC GTGA	1,000	SEQ ID NO: 84
	N918080	TACTCGTGAGG GTCCATCTATG ACCTATCCTGT GTTCTTTACTA GCGAAA	1,000	SEQ ID NO: 85

In addition, in tables 1 to 7, "Linkage group" represents the number given to each group among a plurality of linkage groups specified by QTL analysis. In tables 1 to 7, "Marker name" represents the name given to each marker originally obtained in the present invention. In tables 1 to 7, "Signal threshold" represents a threshold used for determination of the presence or absence of a marker.

In addition, the peak contained in the 18.7-cM region is present in a region sandwiched between a marker (N804607) consisting of the nucleotide sequence shown in SEQ ID NO: 5 and a marker (N815502) consisting of the nucleotide sequence shown in SEQ ID NO: 9. The peak contained in the 39.2-cM region is present in a region sandwiched between a marker (N803928) consisting of the nucleotide sequence shown in SEQ ID NO: 18 and a marker (N826906) consisting of the nucleotide sequence shown in SEQ ID NO: 22. The peak contained in the 19.2-cM region is present in a region sandwiched between a marker (N829378) consisting of the nucleotide sequence shown in SEQ ID NO: 25 and a marker (N821999) consisting of the nucleotide sequence shown in SEQ ID NO: 32. The peak contained in the 32.0-cM region is present in a region sandwiched between a marker (N915070) consisting of the nucleotide sequence shown in SEQ ID NO: 33 and a marker (N918161) consisting of the nucleotide sequence shown in SEQ ID NO: 42. The peak contained in the 39.5-cM region is present in a region sandwiched between a marker (N911103) consisting of the nucleotide sequence shown in SEQ ID NO: 57 and a marker (N918344) consisting of the nucleotide sequence shown in SEQ ID NO: 59. The peak contained in the 53.4-cM region is present in a region sandwiched between a marker (N918761) consisting of the nucleotide sequence shown in SEQ ID NO: 64 and a marker (N900663) consist-

ing of the nucleotide sequence shown in SEQ ID NO: 66. The peak contained in the 38.0-cM region is present in a region sandwiched between a marker (N901524) consisting of the nucleotide sequence shown in SEQ ID NO: 73 and a marker (N915180) consisting of the nucleotide sequence shown in SEQ ID NO: 80.

A continuous nucleic acid region existing in any of 7 regions containing markers shown in tables 1 to 7 can be used as a marker associated with resistance to sugarcane smut. The term "nucleic acid region" used herein refers to a region having a nucleotide sequence having 95% or less, preferably 90% or less, more preferably 80% or less, and most preferably 70% or less identity to a different region present on a sugarcane chromosome. If the identity of a nucleic acid region serving as a marker associated with resistance to sugarcane smut to a different region falls within the above range, the nucleic acid region can be specifically detected according to a standard method. The identity value described herein can be calculated using default parameters and BLAST® or a similar algorithm.

In addition, the base length of a nucleic acid region serving as a marker associated with resistance to sugarcane smut can be at least 8 bases, preferably 15 bases or more, more preferably 20 bases or more, and most preferably 30 bases. If the base length of a nucleic acid region serving as a marker associated with resistance to sugarcane smut falls within the above range, the nucleic acid region can be specifically detected according to a standard method.

In particular, among the 14 types of markers contained in the 18.7-cM region, a marker associated with resistance to sugarcane smut is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 5 and the nucleotide sequence shown in SEQ ID NO: 9. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 5 and the nucleotide sequence shown in SEQ ID NO: 9. In addition, among the 8 types of markers contained in the 39.2-cM region, a marker associated with resistance to sugarcane smut is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 18 and the nucleotide sequence shown in SEQ ID NO: 22. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 18 and the nucleotide sequence shown in SEQ ID NO: 22. Further, among the 10 types of markers contained in the 19.2-cM region, a marker associated with resistance to sugarcane smut is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 25 and the nucleotide sequence shown in SEQ ID NO: 32. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 25 and the nucleotide sequence shown in SEQ ID NO: 30. Furthermore, among the 19 types of markers contained in the 32.0-cM region, a marker associated with resistance to sugarcane smut is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 33 and the nucleotide sequence shown in SEQ ID NO: 42. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 33 and the nucleotide sequence shown in SEQ ID NO: 42. Moreover, among the 11 types of markers contained in the 39.5-cM region, a marker associated with resistance to sugarcane smut is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 57 and the nucleotide sequence shown in SEQ ID NO: 59. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 57 and the nucleotide sequence shown in SEQ ID NO: 59. Among the 10 types of markers contained in the 53.4-cM region, a marker associated with resistance to sugarcane smut is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 64 and the nucleotide sequence shown in SEQ ID NO: 66. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 64 and the nucleotide sequence shown in SEQ ID NO: 66. Among the 13 types of markers contained in the 38.0-cM region, a marker associated with resistance to sugarcane smut is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 73 and the nucleotide sequence shown in SEQ ID NO: 80. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 73 and the nucleotide sequence shown in SEQ ID NO: 80.

In addition, a nucleic acid region containing a single marker selected from among the 85 types of markers shown in tables 1 to 7 can be used as a marker associated with resistance to sugarcane smut. For example, it is preferable to use, as a marker associated with resistance to sugarcane smut, a nucleic acid region containing a marker (N802870) consisting of the nucleotide sequence shown in SEQ ID NO: 6 located closest to the peak position in the 18.7-cM region, a nucleic acid region containing a marker (N826906) consisting of the nucleotide sequence shown in SEQ ID NO: 22 located closest to the peak position in the 39.2-cM region, a nucleic acid region containing a marker (N821999) consisting of the nucleotide sequence shown in SEQ ID NO: 32 located closest to the peak position in the 19.2-cM region, a nucleic acid region containing a marker (N916186) consisting of the nucleotide sequence shown in SEQ ID NO: 35 located closest to the peak position in the 32.0-cM region, a nucleic acid region containing a marker (N918508) consisting of the nucleotide sequence shown in SEQ ID NO: 58 located closest to the peak position in the 39.5-cM region, a nucleic acid region containing a marker (N913735) consisting of the nucleotide sequence shown in SEQ ID NO: 65 located closest to the peak position in the 53.4-cM region, or a nucleic acid region containing a marker (N901163) consisting of the nucleotide sequence shown in SEQ ID NO: 74 located closest to the peak position in the 38.0-cM region. In such case, the nucleotide sequence of a nucleic acid region containing the marker can be specified by flanking sequence analysis such as inverse PCR analysis using primers designed based on the nucleotide sequence of such marker.

Further, as a marker associated with resistance to sugarcane smut, any of the above 85 types of markers can be directly used. Specifically, one or more type(s) of markers selected from among the 85 types of such markers can be directly used as a marker associated with resistance to sugarcane smut. For example, it is preferable to use, as a marker associated with resistance to sugarcane smut, a marker (N802870) consisting of the nucleotide sequence shown in SEQ ID NO: 6 located closest to the peak position in the 18.7-cM region, a marker (N826906) consisting of the nucleotide sequence shown in SEQ ID NO: 22 located closest to the peak position in the 39.2-cM region, a marker (N821999) consisting of the nucleotide sequence shown in SEQ ID NO: 32 located closest to the peak position in the 19.2-cM region, a marker (N916186) consisting of the nucleotide sequence shown in SEQ ID NO: 35 located

closest to the peak position in the 32.0-cM region, a marker (N918508) consisting of the nucleotide sequence shown in SEQ ID NO: 58 located closest to the peak position in the 39.5-cM region, a marker (N913735) consisting of the nucleotide sequence shown in SEQ ID NO: 65 located closest to the peak position in the 53.4-cM region, or a marker (N901163) consisting of the nucleotide sequence shown in SEQ ID NO: 74 located closest to the peak position in the 38.0-cM region.

<Sugarcane Marker Identification>

As described above, markers associated with resistance to sugarcane smut were identified from among 3004 markers and 4569 markers originally obtained from sugarcane chromosomes in the present invention. The 3004 markers and the 4569 markers are described below. Upon identification of these markers, a DNA microarray can be used according to the method disclosed in JP Patent Application No. 2009-283430.

Specifically, the 3004 markers and the 4569 markers originally obtained from sugarcane chromosomes are used with a DNA microarray having probes designed by the method disclosed in JP Patent Application No. 2009-283430. The method for designing probes as shown in FIG. **1** is described below. First, genomic DNA is extracted from sugarcane (step 1a). Next, the extracted genomic DNA is digested with a single or a plurality of restriction enzyme(s) (step 1b). In addition, in the example shown in FIG. **1**, 2 types of restriction enzymes illustrated as restriction enzymes A and B are used (in the order of A first and then B) to digest genomic DNA. The restriction enzymes used herein are not particularly limited. However, examples of restriction enzymes that can be used include PstI, EcoRI, HindIII, BstNI, HpaII, and HaeIII. In particular, restriction enzymes can be adequately selected in consideration of the frequency of appearance of recognition sequences such that a genomic DNA fragment having a base length of 20 to 10000 can be obtained when genomic DNA is completely digested. In addition, when a plurality of restriction enzymes are used, it is preferable for a genomic DNA fragment obtained after the use of all restriction enzymes to have a base length of 200 to 6000. Further, when a plurality of restriction enzymes are used, the order in which restriction enzymes are subjected to treatment is not particularly limited. In addition, a plurality of restriction enzymes may be used in an identical reaction system if they are treated under identical conditions (e.g., solution composition and temperature). Specifically, in the example shown in FIG. **1**, genomic DNA is digested using restriction enzymes A and B in such order. However, genomic DNA may be digested by simultaneously using restriction enzymes A and B in an identical reaction system. Alternatively, genomic DNA may be digested using restriction enzymes B and A in such order. Further, 3 or more restriction enzymes may be used.

Next, adapters are bound to a genomic DNA fragment subjected to restriction enzyme treatment (step 1c). The adapter used herein is not particularly limited as long as it can be bound to both ends of a genomic DNA fragment obtained by the above restriction enzyme treatment. For example, it is possible to use, as an adapter, an adapter having a single strand complementary to a protruding end (sticky end) formed at each end of genomic DNA by restriction enzyme treatment and a primer binding sequence to which a primer used upon amplification treatment as described in detail below can hybridize. In addition, it is also possible to use, as an adapter, an adapter having a single strand complementary to the above protruding end (sticky end) and a restriction enzyme recognition site that is incorporated into a vector upon cloning.

In addition, when genomic DNA is digested using a plurality of restriction enzymes, a plurality of adapters corresponding to the relevant restriction enzymes can be prepared and used. Specifically, it is possible to use a plurality of adapters having single strands complementary to different protruding ends formed upon digestion of genomic DNA with a plurality of restriction enzymes. Here, a plurality of adapters corresponding to a plurality of restriction enzymes each may have a common primer binding sequence such that a common primer can hybridize to each such adapter. Alternatively, they may have different primer binding sequences such that different primers can separately hybridize thereto.

Further, when genomic DNA is digested using a plurality of restriction enzymes, it is possible to prepare and use, as an adaptor, adapter(s) corresponding to one or a part of restriction enzyme(s) selected from among a plurality of the used restriction enzymes.

Next, a genomic DNA fragment to both ends of which adapters have been added is amplified (step 1d). When an adapter having a primer binding sequence is used, the genomic DNA fragment can be amplified using a primer that can hybridize to the primer binding sequence. Alternatively, a genomic DNA fragment to which an adapter has been added is cloned into a vector using the adapter sequence. The genomic DNA fragment can be amplified using primers that can hybridize to specific regions of the vector. In addition, as an example, PCR can be used for a genomic DNA fragment amplification reaction using primers.

When genomic DNA is digested using a plurality of restriction enzymes and a plurality of adapters corresponding to the relevant restriction enzymes are ligated to genomic DNA fragments, the adapters are ligated to all genomic DNA fragments obtained by treatment with a plurality of restriction enzymes. In this case, all the obtained genomic DNA fragments can be amplified by carrying out a nucleic acid amplification reaction using primer binding sequences contained in adapters.

Alternatively, when genomic DNA is digested using a plurality of restriction enzymes, followed by ligation of adapter(s) corresponding to one or a part of restriction enzyme(s) selected from among a plurality of the used restriction enzymes to genomic DNA fragments, among the obtained genomic DNA fragments, a genomic DNA fragment to both ends of which the selected restriction enzyme recognition sequences have been ligated can be exclusively amplified.

Next, the nucleotide sequence of the amplified genomic DNA fragment is determined (step 1e). Then, one or more region, which has a base length shorter than the base length of the genomic DNA fragment and corresponds to at least a partial region of the genomic DNA fragment, is specified. Sugarcane probes are designed using at least one of the thus specified regions (step 1f). A method for determining the nucleotide sequence of a genomic DNA fragment is not particularly limited. A conventionally known method using a DNA sequencer applied to the Sanger method or the like can be used. For example, a region to be designed herein has a 20- to 100-base length, preferably a 30- to 90-base length, and more preferably a 50- to 75-base length as described above.

A DNA microarray can be produced by designing many probes using genomic DNA extracted from sugarcane as described above and synthesizing an oligonucleotide having a desired nucleotide sequence on a support based on the

nucleotide sequence of the designed probe. With the use of a DNA microarray prepared as described above, the 3004 markers and the 4569 markers, including the above 85 types of markers associated with resistance to sugarcane smut shown in SEQ ID NOS: 1 to 85, can be identified.

More specifically, the present inventors obtained signal data of known sugarcane varieties (NiF8 and Ni9) and a progeny line (line 191) obtained by crossing the varieties with the use of the DNA microarray described above. Then, genotype data were obtained based on the obtained signal data. Based on the obtained genotype data, chromosomal marker position information was obtained by calculation using the gene distance function (Kosambi) and the AntMap genetic map creation software (Iwata H, Ninomiya S (2006) AntMap: constructing genetic linkage maps using an ant colony optimization algorithm, Breed Sci 56: 371-378). Further, a genetic map datasheet was created based on the obtained marker position information using Mapmaker/EXP ver. 3.0 (A Whitehead Institute for Biomedical Research Technical Report, Third Edition, January, 1993). As a result, the 3004 markers and the 4569 markers, including the aforementioned 85 types of markers associated with resistance to sugarcane smut shown in SEQ ID NOS: 1 to 85, were identified.

<Use of Markers Associated with Resistance to Sugarcane Smut>

The use of markers associated with resistance to sugarcane smut makes it possible to determine whether a sugarcane progeny line or the like, which has a phenotype exhibiting unknown smut resistance, is a line having a phenotype showing the improvement of smut resistance. The expression "the use of markers associated with resistance to sugarcane smut" used herein indicates the use of a DNA microarray having probes corresponding to markers associated with resistance to sugarcane smut in one embodiment. The expression "probes corresponding to markers associated with resistance to sugarcane smut" indicates oligonucleotides that can specifically hybridize under stringent conditions to markers associated with resistance to sugarcane smut defined as above. For instance, such oligonucleotides can be designed as partial or whole regions with base lengths of at least 10 continuous bases, continuous bases, 20 continuous bases, 25 continuous bases, 30 continuous bases, 35 continuous bases, 40 continuous bases, 45 continuous bases, or 50 or more continuous bases of the nucleotide sequences or complementary strands thereof of markers associated with resistance to sugarcane smut defined as above. In addition, a DNA microarray having such probes may be any type of microarray, such as a microarray having a planar substrate comprising glass, silicone, or the like as a carrier, a bead array comprising microbeads as carriers, or a three-dimensional microarray having an inner wall comprising hollow fibers to which probes are fixed.

The use of a DNA microarray prepared as described above makes it possible to determine whether a sugarcane line such as a progeny line or the like, which has a phenotype exhibiting unknown smut resistance, is a line having a phenotype showing the improvement of smut resistance. In addition, in the case of a method other than the above method involving the use of a DNA microarray, it is also possible to determine whether a sugarcane line, which has a phenotype exhibiting unknown smut resistance, is a line having a trait characterized by the improvement of smut resistance by detecting the above markers associated with resistance to sugarcane smut by a conventionally known method.

The method involving the use of a DNA microarray is described in more detail. As shown in FIG. **2**, first, genomic DNA is extracted from a sugarcane sample. In this case, a sugarcane sample is a sugarcane line such as a sugarcane progeny line, which has a phenotype exhibiting unknown smut resistance, and/or a sugarcane line used as a parent for producing a progeny line, and thus which can be used as a subject to be determined whether to have a trait characterized by the improvement of smut resistance or not. In addition, it is also possible to evaluate smut resistance in a sample plant which is a non-sugarcane plant such as a graminaceous plant (e.g., *Sorghum* or *Erianthus*).

Next, a plurality of genomic DNA fragments are prepared by digesting the extracted genomic DNA with restriction enzymes used for preparing the DNA microarray. Then, the obtained genomic DNA fragments are ligated to adapters used for preparation of the DNA microarray. Subsequently, the genomic DNA fragments, to both ends of which adapters have been added, are amplified using primers employed for preparation of the DNA microarray. Accordingly, sugarcane-sample-derived genomic DNA fragments corresponding to the genomic DNA fragments amplified in step 1d upon preparation of the DNA microarray can be amplified.

In this step, among the genomic DNA fragments to which adapters have been added, specific genomic DNA fragments may be selectively amplified. For instance, in a case in which a plurality of adapters corresponding to a plurality of restriction enzymes are used, genomic DNA fragments to which specific adapters have been added can be selectively amplified. In addition, when genomic DNA is digested with a plurality of restriction enzymes, genomic DNA fragments to which adapters have been added can be selectively amplified by adding adapters only to genomic DNA fragments that have protruding ends corresponding to specific restriction enzymes among the obtained genomic DNA fragments. Thus, specific DNA fragment concentration can be increased by selectively amplifying the specific genomic DNA fragments.

Thereafter, amplified genomic DNA fragments are labeled. Any conventionally known substance may be used as a labeling substance. Examples of a labeling substance that can be used include fluorescent molecules, dye molecules, and radioactive molecules. In addition, this step can be omitted using a labeled nucleotide in the step of amplifying genomic DNA fragments. This is because when genomic DNA fragments are amplified using a labeled nucleotide in the amplification step, amplified DNA fragments can be labeled.

Next, labeled genomic DNA fragments are allowed to come into contact with the DNA microarray under certain conditions such that probes fixed to the DNA microarray hybridize to the labeled genomic DNA fragments. At such time, preferably, highly stringent conditions are provided for hybridization. Under highly stringent conditions, it becomes possible to determine with high accuracy whether or not markers associated with resistance to sugarcane smut are present in a sugarcane sample. In addition, stringent conditions can be adjusted based on reaction temperature and salt concentration. That is, an increase in temperature or a decrease in salt concentration results in more stringent conditions. For example, when a probe having a length of 50 to 75 bases is used, the following more stringent conditions can be provided as hybridization conditions: 40 degrees C. to 44 degrees C.; 0.21 SDS; and 6×SSC.

In addition, hybridization between labeled genomic DNA fragments and probes can be confirmed by detecting a labeling substance. Specifically, after the above hybridiza-

tion reaction of labeled genomic DNA fragments and probes, unreacted genomic DNA fragments and the like are washed, and the labeling substance bound to each genomic DNA fragment specifically hybridizing to a probe is observed. For instance, in a case in which the labeling substance is a fluorescent material, the fluorescence wavelength is detected. In a case in which the labeling substance is a dye molecule, the dye wavelength is detected. More specifically, apparatuses such as fluorescent detectors and image analyzers used for conventional DNA microarray analysis can be used.

As described above, it is possible to determine whether or not a sugarcane sample has the above markers associated with resistance to sugarcane smut with the use of the DNA microarray. Here, as described above, as the marker associated with resistance to sugarcane smut, a marker linked to a trait characterized by the improvement of smut resistance and a marker linked to a trait characterized by the reduction of smut resistance are provided. Markers associated with resistance to sugarcane smut designed based on the three aforementioned regions identified in tables 2, 4, and 7 are linked to a trait characterized by the improvement of smut resistance. Meanwhile, markers associated with resistance to sugarcane smut designed based on the four aforementioned regions identified in tables 1, 3, 5, and 6 are linked to a trait characterized by the reduction of smut resistance.

Therefore, if any one of the markers associated with resistance to sugarcane smut designed based on the three aforementioned regions identified in tables 2, 4, and 7 is present in a sugarcane sample, it is possible to determine that the sample is of a variety with improved smut resistance. Further, if any one of the markers associated with resistance to sugarcane smut designed based on the four aforementioned regions identified in tables 1, 3, 5, and 6 is absent in a sugarcane sample, it is possible to determine that the sample is of a variety with improved smut resistance. Preferably, if any one of the markers associated with resistance to sugarcane smut designed based on the three aforementioned regions identified in tables 2, 4, and 7 is present in a sugarcane sample, and if any one of the markers associated with resistance to sugarcane smut designed based on the four aforementioned regions identified in tables 1, 3, 5, and 6 is absent in the sugarcane sample, it is possible to determine with high accuracy that the sample is of a variety with improved smut resistance.

In particular, according to the method described above, it is not necessary to cultivate sugarcane samples to such an extent that determination using an actual smut resistance test becomes possible. For instance, seeds of a progeny line or a young seedling obtained as a result of germination of such seeds can be used. Therefore, the area of a field used for cultivation of sugarcane samples and other factors such as cost of cultivation can be significantly reduced with the use of the markers associated with resistance to sugarcane smut. In addition, the use of markers associated with resistance to sugarcane smut makes it possible to reduce the cost of facilities such as a large-scale special-purpose greenhouse, a special-purpose field, or isolation facility from an external environment, without the need to actually cause infection with a causative microorganism of smut (*Ustilago scitaminea*).

In particular, when a novel sugarcane variety is created, it is preferable to produce several tens of thousands of seedlings via crossing and then to identify a novel sugarcane variety using markers associated with resistance to sugarcane smut prior to or instead of seedling selection. The use of such markers associated with resistance to sugarcane smut makes it possible to significantly reduce the number of excellent lines that need to be cultivated in an actual field. This allows drastic reduction of time-consuming efforts and the cost required to create a novel sugarcane variety.

Alternatively, upon creation of a new sugarcane variety, firstly, it may be determined whether or not a marker associated with resistance to sugarcane smut is present in a parent variety used for crossing, thereby allowing selection of a parent variety with excellent smut resistance. It can be expected that a progeny line with excellent smut resistance will be obtained with high frequency by creating a parent variety with excellent smut resistance on a priority basis. The use of such marker(s) associated with resistance to sugarcane smut makes it possible to significantly reduce the number of excellent lines that need to be cultivated. This allows drastic reduction of time-consuming efforts and the cost required to create a novel sugarcane variety.

EXAMPLES

The present invention is hereafter described in greater detail with reference to the following examples, although the technical scope of the present invention is not limited thereto.

1. Production of DNA Microarray Probes

(1) Materials

The following varieties were used: sugarcane varieties: NiF8, Ni9, US56-15-8, POJ2878, Q165, R570, Co290 and B3439; closely-related sugarcane wild-type varieties: Glagah Kloet, Chunee, Natal Uba, and Robustum 9; and *Erianthus* varieties: IJ76-349 and JW630.

(2) Restriction Enzyme Treatment

Genomic DNA was extracted from each of the above sugarcane varieties, closely-related sugarcane wild-type varieties, and *Erianthus* varieties using DNeasy Plant Mini Kits (Qiagen). Genomic DNAs (750 ng each) were treated with a PstI restriction enzyme (NEB; 25 units) at 37 degrees C. for 2 hours. A BstNI restriction enzyme (NEB; 25 units) was added thereto, followed by treatment at 60 degrees C. for 2 hours.

(3) Adapter Ligation

PstI sequence adapters (5'-CACGATGGATCCAGTGCA-3' (SEQ ID NO: 86) and 5'-CTGGATCCATCGTGCA-3' (SEQ ID NO: 87)) and T4 DNA Ligase (NEB; 800 units) were added to the genomic DNA fragments treated in (2) (120 ng each), and the obtained mixtures were subjected to treatment at 16 degrees C. overnight. Thus, the adapters were selectively added to genomic DNA fragments having PstI recognition sequences at both ends thereof among the genomic DNA fragments treated in (2).

(4) PCR Amplification

A PstI sequence adapter recognition primer (5'-GATGGATCCAGTGCAG-3' (SEQ ID NO: 88)) and Taq polymerase (TAKARA; PrimeSTAR; 1.25 units) were added to the genomic DNA fragment (15 ng) having the adaptors obtained in (3). Then, the genomic DNA fragment was amplified by PCR (treatment at 98 degrees C. for 10 seconds, 55 degrees C. for 15 seconds, 72 degrees C. for 1 minute for 30 cycles, and then at 72 degrees C. for 3 minutes, followed by storage at 4 degrees C.).

(5) Genome Sequence Acquisition

The nucleotide sequence of the genomic DNA fragment subjected to PCR amplification in (4) was determined by the Sanger method. In addition, information on a nucleotide sequence sandwiched between PstI recognition sequences was obtained based on the total *sorghum* genome sequence information contained in the genome database (Gramene).

(6) Probe Design and DNA Microarray Production

50- to 75-bp probes were designed based on the genome sequence information in (5). Based on the nucleotide sequence information of the designed probes, a DNA microarray having the probes was produced.

2. Acquisition of Signal Data Using a DNA Microarray

(1) Materials

Sugarcane varieties/lines (NiF8 and Ni9) and the progeny line (line 191) were used.

(2) Restriction Enzyme Treatment

Genomic DNAs were extracted from NiF8, Ni9, and the progeny line (line 191) using DNeasy Plant Mini Kits (Qiagen). Genomic DNAs (750 ng each) were treated with a PstI restriction enzyme (NEB; 25 units) at 37 degrees C. for 2 hours. Then, a BstNI restriction enzyme (NEB; 25 units) was added thereto, followed by treatment at 60 degrees C. for 2 hours.

(3) Adapter Ligation

PstI sequence adapters (5'-CACGATGGATCCAGTGCA-3' (SEQ ID NO: 86) and 5'-CTGGATCCATCGTGCA-3' (SEQ ID NO: 87)) and T4 DNA Ligase (NEB; 800 units) were added to the genomic DNA fragments treated in (2) (120 ng each), and the obtained mixtures were treated at 16 degrees C. overnight. Thus, the adaptors were selectively added to a genomic DNA fragment having PstI recognition sequences at both ends thereof among the genomic DNA fragments treated in (2).

(4) PCR Amplification

A PstI sequence adapter recognition primer (5'-GATG-GATCCAGTGCAG-3' (SEQ ID NO: 88)) and Taq polymerase (TAKARA; PrimeSTAR; 1.25 units) were added to the genomic DNA fragment (15 ng) having the adapters obtained in (3). Then, the genomic DNA fragment was amplified by PCR (treatment at 98 degrees C. for 10 seconds, 55 degrees C. for 15 seconds, 72 degrees C. for 1 minute for 30 cycles, and then 72 degrees C. for 3 minutes, followed by storage at 4 degrees C.).

(5) Labeling

The PCR amplification fragment obtained in (4) above was purified with a column (Qiagen). Cy3-labeled 9mers (TriLink; 1 O.D.) was added thereto. The resultant was treated at 98 degrees C. for 10 minutes and allowed to stand still on ice for 10 minutes. Then, Klenow (NEB; 100 units) was added thereto, followed by treatment at 37 degrees C. for 2 hours. Thereafter, a labeled sample was prepared by ethanol precipitation.

(6) Hybridization/Signal Detection

The labeled sample obtained in (5) was subjected to hybridization using the DNA microarray prepared in 1 above in accordance with the NimbleGen Array User's Guide. Signals from the label were detected.

3. Identification of QTL for Sugarcane Smut Resistance and Development of Markers

(1) Creation of Genetic Map Datasheet

Genotype data of possible 3004 markers and 4569 markers were obtained based on the signal data detected in 2 above of the NiF8 and Ni9 sugarcane varieties and the progeny line (line 191). Based on the obtained genotype data, chromosomal marker position information was obtained by calculation using the gene distance function (Kosambi) and the AntMap genetic map creation software (Iwata H, Ninomiya S (2006) AntMap: constructing genetic linkage maps using an ant colony optimization algorithm, Breed Sci 56: 371-378). Further, a genetic map datasheet was created based on the obtained marker position information using Mapmaker/EXP ver. 3.0 (A Whitehead Institute for Biomedical Research Technical Report, Third Edition, January, 1993).

(2) Acquisition of Smut Resistance Data

From Oct. 26 to 28, 2009, stalks of NiF8, Ni9, and the 191 hybrid progeny line were harvested. They were subjected to treatment for stimulating germination at room temperature and high humidity for 2 to 3 days, followed by wound inoculation with smut spores. For wound inoculation, wounds were made on both sides of buds (6 wounds in total; approximately 4.0 mm in depth), and then a spore suspension (10^7 to 10^8 spores/ml) was applied to the wounds using a brush. Smut spores in the spore suspension were collected from smut whips of Ni9 stocks naturally infected with smut, which were cultivated in Okinawa in 2009. Seedlings subjected to wound inoculation were cultivated for 2 to 3 days at room temperature and high humidity and planted in nursery boxes from October 30 to Nov. 1, 2009 (40 buds/box, 2 boxes/line). The planted seedlings were cultivated at high humidity in a greenhouse until Sep. 2, 2010. The degree of the development of smut was investigated by counting, as the number of affected seedlings, the number of seedlings showing a symptom of smut, which is the outgrowth of a smut whip from the apex of a stalk. After the count of the affected seedlings, the plant bodies of affected seedlings were harvested at the ground level so that they could be removed. The number of seedlings affected with smut was investigated on Jun. 23, Jul. 21, Aug. 18, and Sep. 2, 2010 for a total of four instances. The incidence of smut was calculated as a percentage of the number of germinating stocks (excluding stocks killed by non-smut causes) accounted for by the number of affected stocks. FIGS. **3**, **4**, **5**, and **6** show the study results of Jun. 23, 2010, the study results of Jul. 21, 2010, the study results of Aug. 18, 2010, and the study results of Sep. 2, 2010, respectively.

(3) Quantitative Trait (Quantitative Trait Loci: QTL) Analysis

Based on the genetic map datasheet obtained in (1) above and the smut resistance data obtained in (2) above, QTL analysis was carried out by the composite interval mapping (CIM) method using the QTL Cartographer gene analysis software (Wang S., C. J. Basten, and Z.-B. Zeng (2010). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, N.C.). Upon analysis, the LOD threshold was determined to be 2.5. As a result, as shown in FIGS. **7** to **12**, the presence of QTL regarding sugarcane smut resistance was confirmed in the following seven ranges: the range between markers N827337 and N821515 present in the 5th linkage group (August 18), the range between markers N826561 and N826906 present in the 17th linkage group (June 23, July 21, and August 18), and the range between markers N816552 and N821999 present in the 40th linkage group (July 21, August 18, and September 2) of the NiF8 sugarcane variety; the range between markers N915070 and N920207 present in the 1st linkage group (July 21, August 18, and September 2), the range between markers N914284 and N916129 present in the 13th linkage group (July 21, August 18, and September 2), and the range between markers N901177 and N900802 (June 23) and the range between markers N901524 and N918080 (June 23 and July 21) present in the 14th linkage group of the Ni9 sugarcane variety. Specifically, peaks exceeding the LOD threshold were observed in the above seven ranges. It was possible to specify the obtained peaks as shown in table 8, suggesting the presence of a causative gene (or a group of causative genes) having the function of causing the improvement of smut resistance at the each peak positions. In addition, the "Effect (%)" column in table 8 indicates an increase or a decrease in the incidence of smut. Therefore, if the value of "Effect (%)" is negative, it means that the QTL (quantitative trait locus) is linked to a trait characterized by the improvement of smut resistance. If the value of "Effect (%)" is positive, it means that the QTL is linked to a trait characterized by the reduction of smut resistance.

TABLE 8

Variety	Linkage group	Investigation date	Position (cM)	Range (cM)	Close marker	LOD score	Effect (%)
NiF8	5	8/18	3.8	18.7	N827337-N821515	2.6	8.2
NiF8	17	6/23	94.3	39.2	N826561-N826906	6.5	−16.5
NiF8	17	7/21	94.3	39.2	N826561-N826906	3.6	−10.1
NiF8	17	8/18	94.3	39.2	N826561-N826906	3.5	−9.7
NiF8	40	7/21	34.1	19.2	N816552-N821999	3.3	9.6
NiF8	40	8/18	34.1	19.2	N816552-N821999	3.0	8.9
NiF8	40	9/2	34.1	19.2	N816552-N821999	3.4	8.4
Ni9	1	7/21	5.5	32.0	N915070-N920207	2.8	−8.7
Ni9	1	8/18	5.5	32.0	N915070-N920207	2.6	−8.3
Ni9	1	9/2	5.5	32.0	N915070-N920207	3.3	−8.5
Ni9	13	7/21	12.9	39.5	N914284-N916129	2.6	8.3
Ni9	13	8/18	12.9	39.5	N914284-N916129	2.6	8.3
Ni9	13	9/2	12.9	39.5	N914284-N916129	2.6	7.4
Ni9	14_1	6/23	97.3	53.4	N901178-N900802	2.6	15.2
Ni9	14_2	6/23	137.7	38.0	N901524-N918080	5.2	−21.3
Ni9	14_2	7/21	137.7	38.0	N901524-N918080	4.7	−16.2

As shown in FIGS. **7** to **12**, a marker located in the vicinity of the peak is inherited in linkage with a causative gene (or a group of causative genes) having the function of causing the improvement or reduction of smut resistance. This shows that the markers can be used as markers associated with resistance to sugarcane smut. Specifically, it has been revealed that the 85 types of markers shown in FIGS. **7** to **12** can be used as markers associated with resistance to sugarcane smut.

In addition, as examples of signals detected in 2 (6) above, table 9 shows signal levels of 14 types of markers among markers N827337 to N821515 present in the 5th linkage group of NiF8 for NiF8 and Ni9 and their progeny lines. In particular, the signal levels of N802870 are shown in FIG. **13**.

TABLE 9

Linkage group	Marker name	NiF8	Ni9	F1											
NiF8_5	N827337	1,629	529	1,354	344	439	403	1,330	1,593	1,823	1,495	1,717	512	495	739
	N802879	4,193	393	2,706	370	347	372	1,484	2,319	1,707	1,897	1,803	365	518	389
	N804818	3,093	591	2,173	531	494	408	2,480	3,233	3,589	4,092	4,075	533	635	613
	N816296	1,489	379	1,440	510	358	342	1,445	1,822	1,671	1,664	1,691	355	396	336
	N804607	2,125	375	1,454	393	361	394	1,258	1,266	1,422	1,416	1,311	660	382	495
	N802870	5,496	828	4,275	377	412	444	5,198	4,496	4,195	4,631	4,207	446	655	361
	N813249	6,034	778	4,329	553	498	764	4,208	3,754	3,864	3,749	3,330	627	711	414
	N813609	2,821	701	2,178	750	901	869	2,820	3,222	3,729	2,888	3,552	566	945	840
	N815502	2,044	481	2,452	806	493	436	2,390	2,587	2,088	2,211	2,088	493	640	425
	N815101	2,055	446	2,660	549	419	344	3,184	2,673	3,153	3,105	3,116	504	347	346
	N823481	2,096	509	1,200	457	487	393	1,629	1,460	1,870	1,925	1,920	528	585	402
	N801028	6,877	907	5,694	886	799	651	5,083	3,359	3,578	4,019	4,197	792	377	930
	N810798	5,506	633	5,171	823	608	513	5,720	4,545	5,463	6,279	5,907	561	847	775
	N821515	3,768	819	3,190	790	489	418	4,899	3,485	3,282	3,331	3,603	553	921	515

Signal levels of 14 types of markers were found to be remarkably high for progeny lines exhibiting reduction of smut resistance among the linkage groups present in NiF8. These results also revealed that 14 types of markers among markers N827337 to N821515 present in the 5th linkage group can be used as markers associated with resistance to sugarcane smut.

Similarly, table 10 lists signal levels of 8 types of markers among markers N826561 to N826906 present in the 17th linkage group of NiF8 in NiF8 and Ni9 and the progeny lines. In particular, the signal levels of N827136 are shown in FIG. **14**.

TABLE 10

Linkage group	Marker name	NiF8	Ni9	F1											
NiF8_17	N826561	1,977	525	462	1,514	574	1,629	744	864	1,136	1,415	528	1,752	453	1,350
	N827136	5,717	514	390	3,279	405	2,898	423	433	4,050	3,633	445	3,857	547	3,723
	N826325	1,620	404	421	1,103	358	1,102	381	408	1,081	1,409	408	1,458	381	1,317
	N803928	2,082	403	390	1,517	427	1,875	412	426	1,696	1,520	393	1,743	322	1,620
	N822568	3,592	501	753	2,556	466	2,502	360	506	2,159	2,941	425	2,733	571	2,580
	N829026	1,766	540	432	1,656	452	1,759	396	656	2,159	2,325	456	1,906	558	2,041
	N815300	3,128	669	708	1,951	974	2,189	460	439	2,271	1,981	687	2,039	372	2,028
	N826906	2,339	447	407	1,704	754	2,139	679	485	2,122	2,554	361	1,915	480	2,281

Signal levels of 8 types of markers were found to be remarkably high for progeny lines exhibiting excellent smut resistance among the linkage groups present in NiF8. These results also revealed that 8 types of markers among markers N826561 to N826906 present in the 17th linkage group can be used as markers associated with resistance to sugarcane smut.

Similarly, table 11 lists signal levels of 10 types of markers among markers N816552 to N821999 present in the 40th linkage group of NiF8 in NiF8 and Ni9 and the progeny lines. In particular, the signal levels of N812680 are shown in FIG. **15**.

TABLE 11

Linkage group	Marker name	NiF8	Ni9	F1											
NiF8_40	N816552	2,731	770	630	622	845	3,929	4,570	4,215	3,666	4,651	4,394	900	719	747
	N827448	2,470	450	357	391	340	2,664	3,031	2,325	2,650	2,035	2,473	376	564	467
	N829378	3,344	609	443	505	453	1,508	2,045	2,319	1,428	1,093	1,506	752	603	497
	N829404	2,700	752	723	758	771	2,641	2,295	2,645	2,128	2,792	3,259	523	748	794
	N828725	2,053	461	548	542	433	1,860	2,282	2,130	1,645	2,066	1,435	496	368	453
	N812680	7,958	377	309	322	343	6,921	7,267	5,468	5,905	9,015	8,278	317	378	490
	N811688	4,885	410	680	954	496	3,324	4,237	3,073	2,636	2,919	3,384	520	471	649
	N819703	4,612	736	617	820	633	4,054	5,138	3,636	5,267	5,107	3,622	761	648	907
	N815648	3,391	471	472	550	363	2,902	3,116	2,694	2,747	3,836	3,554	393	680	483
	N821999	3,255	678	427	422	427	2,959	2,237	3,538	2,036	2,680	3,002	904	413	401

Signal levels of 10 types of markers were found to be remarkably high for progeny lines exhibiting reduction of smut resistance among the linkage groups present in NiF8. These results also revealed that 10 types of markers among markers N816552 to N821999 present in the 40th linkage group can be used as markers associated with resistance to sugarcane smut.

Similarly, table 12 lists signal levels of 19 types of markers among markers N915070 to N920207 present in the 1st linkage group of Ni9 in NiF8 and Ni9 and the progeny lines. In particular, the signal levels of N916081 are shown in FIG. **16**.

TABLE 12

Linkage group	Marker name	NiF8	Ni9	F1											
Ni9_1	N915070	424	1,195	418	1,393	1,122	1,717	480	1,356	1,359	1,707	370	424	424	403
	N915209	560	1,796	435	2,840	1,778	3,016	601	2,376	2,361	3,451	446	541	462	484
	N916186	496	2,002	403	2,447	1,808	1,571	415	2,608	1,723	2,687	406	671	466	432
	N902342	372	1,245	349	1,049	1,003	1,045	420	1,062	1,346	2,206	333	352	401	398
	N919949	625	1,459	406	2,169	1,942	2,723	738	1,679	2,360	3,490	650	680	444	572
	N920597	450	4,702	399	5,028	3,819	5,583	348	6,733	4,669	7,196	436	431	393	537
	N916081	516	13,678	954	16,011	14,893	10,082	634	10,528	10,441	11,232	504	785	435	604
	N902047	955	5,233	658	4,400	3,853	4,711	825	3,378	5,336	6,194	581	992	555	854
	N916874	491	3,320	486	2,511	2,869	3,276	708	2,304	3,046	4,073	416	791	409	430
	N918161	438	2,109	411	1,989	1,892	2,109	397	1,690	2,193	2,643	406	610	398	335
	N918536	372	1,059	508	1,229	1,293	1,368	487	1,253	1,704	1,967	511	423	395	381
	N901676	648	1,534	702	2,407	1,395	1,389	705	1,590	1,820	1,918	521	577	483	582
	N919743	635	2,361	437	1,703	1,731	1,990	471	2,385	2,076	3,665	568	417	399	398
	N901176	697	5,017	408	3,009	5,027	5,059	820	5,316	3,362	3,347	764	454	715	420
	N916035	757	4,444	684	3,088	3,803	3,576	580	4,310	4,270	4,272	448	585	485	581
	N921010	521	5,630	448	6,214	5,012	7,792	909	5,074	4,902	5,904	557	658	559	611
	N915635	424	7,875	538	12,542	10,900	15,388	568	9,698	10,501	14,732	391	505	400	469

TABLE 12-continued

Linkage group	Marker name	NiF8	Ni9	F1										
	N901348	493	3,188	558	6,692	7,451	6,466	605	3,553	7,406	2,655	659	584	638	438
	N920207	421	5,291	349	4,550	4,857	6,695	385	1,962	3,567	11,697	449	478	416	450

Signal levels of 19 types of markers were found to be remarkably high for progeny lines exhibiting excellent smut resistance among the linkage groups present in Ni9. These results also revealed that 19 types of markers among markers N915070 to N920207 present in the 1st linkage group can be used as markers associated with resistance to sugarcane smut.

Similarly, table 13 lists signal levels of 11 types of markers among markers N914284 to N916129 present in the 13th linkage group of Ni9 in NiF8 and Ni9 and the progeny lines. In particular, the signal levels of N919839 are shown in FIG. **17**.

TABLE 13

Linkage group	Marker name	NiF8	Ni9	F1										
Ni9_13	N914284	439	1,102	380	551	1,358	562	1,234	1,463	1,247	877	1,235	492	467	1,342
	N901453	788	1,511	661	638	1,120	560	1,380	2,056	2,671	807	1,415	512	533	1,821
	N900044	688	1,693	646	652	1,600	554	1,632	2,841	2,410	618	1,619	603	577	1,639
	N919839	389	4,719	364	329	4,261	332	4,331	6,387	5,540	360	4,307	352	334	7,400
	N901567	426	2,890	374	399	3,213	526	3,981	5,253	5,212	434	3,364	449	383	4,346
	N911103	500	902	388	424	1,069	414	1,246	2,352	1,465	408	1,036	417	630	1,500
	N918508	686	5,836	513	667	4,963	778	4,317	7,106	5,911	599	4,037	587	547	7,786
	N918344	385	1,970	352	578	1,765	470	2,000	3,298	2,428	409	2,079	426	483	2,488
	N919696	497	2,696	496	427	2,327	685	1,928	2,433	2,370	414	2,043	680	657	2,763
	N916172	471	1,960	448	378	3,025	445	2,990	4,282	3,314	433	2,666	403	445	3,518
	N916129	526	5,026	641	506	3,393	858	3,465	5,094	5,261	478	4,030	383	558	6,621

Signal levels of 11 types of markers were found to be remarkably high for progeny lines exhibiting reduction of smut resistance among the linkage groups present in Ni9. These results also revealed that 11 types of markers among markers N914284 to N916129 present in the 13th linkage group can be used as markers associated with resistance to sugarcane smut.

Similarly, table 14 lists signal levels of 10 types of markers among markers N901178 to N900802 present in the 14th linkage group of Ni9 in NiF8 and Ni9 and the progeny lines. In particular, the signal levels of N918761 are shown in FIG. **18**.

TABLE 14

Linkage group	Marker name	NiF8	Ni9	F1										
Ni9_14_1	N901178	617	1,779	1,250	508	414	1,929	1,698	1,986	470	584	1,104	498	1,454	539
	N918761	607	5,766	6,048	453	631	5,410	4,505	6,440	519	484	2,747	453	4,233	473
	N913735	850	2,996	2,251	557	519	2,576	2,759	3,188	496	555	1,903	461	2,490	651
	N900663	686	3,173	2,014	412	466	2,351	3,156	4,168	475	423	1,810	559	2,534	662
	N918363	477	1,964	1,961	573	481	1,895	2,092	2,809	516	486	2,012	496	2,223	583
	N918213	760	2,319	3,224	882	798	3,485	3,433	4,402	579	507	3,767	678	2,874	509
	N900568	1,040	3,437	3,017	581	368	2,479	3,246	3,387	571	476	2,088	525	1,821	833
	N912523	626	6,398	6,371	476	565	4,799	5,756	7,064	526	813	4,739	424	4,431	541
	N900344	692	5,788	6,366	838	759	3,590	5,674	6,474	588	729	6,622	640	5,622	542
	N900802	717	6,090	6,668	453	537	4,639	6,414	8,043	618	905	5,322	430	6,032	619

Signal levels of 10 types of markers were found to be remarkably high for progeny lines exhibiting reduction of smut resistance among the linkage groups present in Ni9. These results also revealed that 10 types of markers among markers N901178 to N900802 present in the 14th linkage group can be used as markers associated with resistance to sugarcane smut.

Similarly, table 15 lists signal levels of 13 types of markers among markers N901524 to N918080 present in the 14th linkage group of Ni9 in NiF8 and Ni9 and the progeny lines. In particular, the signal levels of N901160 are shown in FIG. **19**.

TABLE 15

Linkage group	Marker name	NiF8	Ni9					F1							
Ni9_14_2	N901524	428	2,677	2,763	425	457	343	2,534	1,954	617	445	3,083	1,291	2,532	674
	N901163	379	3,462	2,099	909	861	632	3,268	3,426	600	380	3,196	3,202	3,260	392
	N911063	575	4,326	5,570	385	466	384	2,738	7,453	398	409	7,625	8,794	2,220	381
	N914692	625	3,592	4,016	811	577	419	3,574	3,955	742	959	3,573	4,756	4,094	714
	N911405	386	1,893	1,692	470	404	411	1,715	2,402	411	437	1,762	2,052	1,660	396
	N913383	580	2,923	2,202	710	421	596	2,256	2,986	821	798	2,768	3,336	2,062	708
	N914112	564	3,387	3,390	825	417	730	1,913	3,528	1,000	987	2,934	3,753	2,600	966
	N915180	537	2,482	2,950	566	485	396	2,590	2,968	438	497	2,242	3,021	3,110	729
	N901160	452	4,333	6,165	375	394	333	4,340	6,432	424	417	7,227	8,545	4,069	359
	N916293	560	2,069	1,908	725	868	520	1,420	3,179	463	517	1,720	2,129	1,681	464
	N916263	414	2,358	1,775	379	348	359	2,136	2,174	522	404	2,041	1,850	2,502	491
	N917579	485	2,335	1,873	501	459	395	2,209	3,210	440	390	2,959	1,816	3,724	378
	N918080	469	1,238	1,171	432	532	361	1,621	1,567	380	402	1,269	1,015	1,940	442

Signal levels of 13 types of markers were found to be remarkably high for progeny lines exhibiting excellent smut resistance among the linkage groups present in Ni9. These results also revealed that 13 types of markers among markers N901524 to N918080 present in the 14th linkage group can be used as markers associated with resistance to sugarcane smut.

All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

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<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 26

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atc 63

<210> SEQ ID NO 27
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 27

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aatctaaatg actaatgaga ccgtgagagc tgcttagctt aatggtgcat ccctttttaa 60

act 63

<210> SEQ ID NO 28
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 28

aagaacactg ctaaggatgg tcacaatttg gaaactgaag ttttatctct ggttcggt 58

<210> SEQ ID NO 29
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 29

aagctgcatc tgattctcat ccaaacctgc tctgctcatt atcattactt cgt 53

<210> SEQ ID NO 30
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 30

ccaaccaaca gcaagaacac caagacgcac ataatgaggc ccatgaagta 50

<210> SEQ ID NO 31
<211> LENGTH: 64
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 31

tttacaccag tgaactgaca aaaaatcgaa gtggtgcggt acataagaac atttacatcc 60

aact 64

<210> SEQ ID NO 32
<211> LENGTH: 73
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 32

gaccaatcta ggaaaaacaa ttgcacaaat gactacattt attatggcaa atcaattttc 60

ttcagtcatt gta 73

<210> SEQ ID NO 33
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 33

atagtctacc tatactggtg ccacaagtca acaagtgatg gcaataccca ttcaaatt 58

<210> SEQ ID NO 34
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 34

tggcaatacc cattcaaatt gcgtcaaatg tgaataaatg gaggtagatg actaacacct 60

ttgtttcaaa a 71

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<210> SEQ ID NO 35
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 35

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<210> SEQ ID NO 36
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 36

ccaaatacct aagtgcactt ttttctgagg ccaaatacct aggttcgaaa gattcgt 57

<210> SEQ ID NO 37
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 37

ccgcctcaaa aggaagtaac acaggaacat gatcatacgg agtagtacta t 51

<210> SEQ ID NO 38
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 38

cttgccggcc gggaccctgc tggcacgatc aagcgactac agtacaatgc 50

<210> SEQ ID NO 39
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 39

caaagaaagc acattaccgc gtatgttacc aacttcctat gttgactatc caaatactg 59

<210> SEQ ID NO 40
<211> LENGTH: 65
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 40

ggattggtct agtacaatct ttattgaaga cgaaagattt atgcatggtg attagttgag 60

cctgt 65

<210> SEQ ID NO 41
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 41

caaatatgac gatggaaata tatagtacta ttaataagac ataacttgca gcatatatta 60

atttcatagg ataag 75

<210> SEQ ID NO 42
<211> LENGTH: 50
<212> TYPE: DNA

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<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 42

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<210> SEQ ID NO 43
<211> LENGTH: 50
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<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 43

cagagaaact gggaacgaaa caggacaata catctgtacg tttggcttgt 50

<210> SEQ ID NO 44
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 44

tccctgtact gtatggtcgc cacaaatgca tattgataga catgtttatg atgtagaatt 60

tgatgtttac a 71

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<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 45

aaatcaataa agaaaggcac gctgaaaata agatggtctg atcgagctcc tgtgtttagt 60

acaa 64

<210> SEQ ID NO 46
<211> LENGTH: 73
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 46

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atcaatcaac taa 73

<210> SEQ ID NO 47
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 47

gccttcttga tctctcagac taagaacata ggcccagagt gaggggaaac 50

<210> SEQ ID NO 48
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 48

cgttcgcttg agcttattag ataaaatcaa tcagcaataa aataatattt ttttctaata 60

aaaatcagca 70

<210> SEQ ID NO 49
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

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<400> SEQUENCE: 49

tttatcagct tcggaaatca gcttgagctg acgaagacat caatcttcta catcagat 58

<210> SEQ ID NO 50
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 50

acatgtatgt gcaaaatatc ttgagaccct ctgctttaac atgcatgtcc ttcacatgt 59

<210> SEQ ID NO 51
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 51

cagctctgtc attgccgcca aacacatatg cgccttcatg cccttctccc 50

<210> SEQ ID NO 52
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 52

agccatcccg cagaggctct tgatgtcctt tgagctgtcc taaaaccact 50

<210> SEQ ID NO 53
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 53

ctatgtgttg ggcttatatg tgatgcatct ttccttttga attcagggta gtgctgata 59

<210> SEQ ID NO 54
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 54

gtgctgatac gccaccagcc gaaacaaatg gtgatagctc tagcgcacag 50

<210> SEQ ID NO 55
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 55

aaatcctgaa ggccgaagcc cgtagacatg ttcaccctag caaacaaagg 50

<210> SEQ ID NO 56
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 56

gcatcggctg gtgctggtag ggataaacct ctgctccgct tgatattttt 50

<210> SEQ ID NO 57
<211> LENGTH: 71
<212> TYPE: DNA

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<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 57

ttcgcttgag ttttatcagc agaattaaca gttatatagc ggtgtttttt ctctcacact 60

aaatcagtaa a 71

<210> SEQ ID NO 58
<211> LENGTH: 71
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 58

cttgcctact tcttgcatag atgcttagtt tacattttac ctgaaattta ttaatatcga 60

tcactacaaa t 71

<210> SEQ ID NO 59
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 59

gaacaaggag catccatata tgtatggcac tttgacattg ttggctatgt ctagctt 57

<210> SEQ ID NO 60
<211> LENGTH: 50
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<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 60

ggaaaagcaa gcagctcgtg tagcaatagt tggcattggc aacagacgcc 50

<210> SEQ ID NO 61
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 61

ggtaaaatta tgcaagttcc cacgaaattt ggcatatgaa agtgccctta aaaattaagg 60

ttt 63

<210> SEQ ID NO 62
<211> LENGTH: 73
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 62

gagcttttat ttatgctaac ctgtaacaat aaattgtctt tgagcatggt ttgtttgatg 60

atctcaatga ccg 73

<210> SEQ ID NO 63
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<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 63

atctacacaa caaatccact gtattagacg attgttatca aatgatcttc cagcaaattg 60

acataatatg acatt 75

<210> SEQ ID NO 64
<211> LENGTH: 66

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<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 64

agaacagggc catcgttgtt agcgtgcgtg ctgtaagttt gatttaattt aaaaaaaata 60

cgtata 66

<210> SEQ ID NO 65
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 65

acgtacaaat gtttgggatg gcagaggaca tgtagtacag ggttgattct tttcaata 58

<210> SEQ ID NO 66
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 66

gcacctcgct cctccttatc aagtttcgat ttctggattt gctgctcttg 50

<210> SEQ ID NO 67
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 67

aaggcgaaca aatgattccc ctcagtgacc tgaacgtaat agtaaaatga tacacact 58

<210> SEQ ID NO 68
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 68

tcgcatgtca gggctgacaa atggctaaaa ccagacggaa gatagacgga 50

<210> SEQ ID NO 69
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 69

aacatcagct tagtctttag aggttatacc tgctgtgcta tttttttttac ttagtgtaca 60

ccattcctga 70

<210> SEQ ID NO 70
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 70

ccttaatcac gcttgtgaaa tatcactcaa accaacaata tcaataccac cattaattat 60

gcttgtgaaa tatgc 75

<210> SEQ ID NO 71
<211> LENGTH: 73
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

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<400> SEQUENCE: 71

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gaatggttgc aga 73

<210> SEQ ID NO 72
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<400> SEQUENCE: 72

atcccatcac aaaggaaaga attgcacaaa caatgacgtg gtacctttaa aagatagaga 60

atggaataga 70

<210> SEQ ID NO 73
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 73

aagcaacaga tgactagaag tacagtgcag gagactccaa cactttacta tattagtaga 60

aga 63

<210> SEQ ID NO 74
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 74

tcttcagttc atatctatca tctatccgtc gctcgtttca tgagacagat caaataagca 60

gat 63

<210> SEQ ID NO 75
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 75

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aggtgtgtgt 70

<210> SEQ ID NO 76
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 76

cgcccaccaa tgcattaccc aatggggtac ccgatgccgc cccattcgca 50

<210> SEQ ID NO 77
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 77

gtgcagggta cccgtcaatg ggctacggct atggccgccc accaatgcat 50

<210> SEQ ID NO 78
<211> LENGTH: 74
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

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<400> SEQUENCE: 78

aagataaatt tacaagcaaa attagaatgt caaataccac aaatattgag agctgtgcct 60

gacaattgag gaga 74

<210> SEQ ID NO 79
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 79

agctgtgcct gacaattgag agtgaacaga gtacatttca tactgcccag 50

<210> SEQ ID NO 80
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 80

tccggagatt acaacgtctt cagtgacgag aacccgaaca gctgctcggt 50

<210> SEQ ID NO 81
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 81

cccctgacac gatatttatt tgccagaatt tatgaattac agccgcattt cgttgtgt 58

<210> SEQ ID NO 82
<211> LENGTH: 68
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 82

ttggcaatca tcgactaatt aggtgtaaaa gattcgtctt gttattttct accaaattat 60

gaaattta 68

<210> SEQ ID NO 83
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 83

tatagggcca gataaaccat gataatcata ggatatttgc agaaatctta aatttctgag 60

attgccaaca gaaga 75

<210> SEQ ID NO 84
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 84

tatggatctt ccagttgatt actgttcttt cgctccgctt tttgcttttt tactcgtga 59

<210> SEQ ID NO 85
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Saccharum officinarum

<400> SEQUENCE: 85

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tactcgtgag ggtccatcta tgacctatcc tgtgttcttt actagcgaaa 50

<210> SEQ ID NO 86
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA

<400> SEQUENCE: 86

cacgatggat ccagtgca 18

<210> SEQ ID NO 87
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA

<400> SEQUENCE: 87

ctggatccat cgtgca 16

<210> SEQ ID NO 88
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic DNA

<400> SEQUENCE: 88

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The invention claimed is:

1. A method for producing a sugarcane line having improved smut resistance, said method comprising:

extracting genomic DNA from a progeny plant obtained from parent plants, at least one of which is a sugarcane plant, and/or a genomic DNA of a parent sugarcane plant;

determining by nucleic acid assay the presence of a marker associated with resistance to sugarcane smut in the obtained genomic DNA, and selecting the progeny plant as a plant having improved smut resistance based on the presence of said marker; and

using the selected plant as a parent plant for crossing, to thereby produce progeny plant(s),

wherein said marker consists of a continuous nucleic acid region in the obtained genomic DNA, and wherein said marker comprises at least 20 continuous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs: 19 to 22.

2. The method for producing a sugarcane line according to claim **1**, wherein a DNA chip that comprises a probe corresponding to the marker associated with resistance to sugarcane smut is used in the determination step.

3. The method for producing a sugarcane line according to claim **1**, wherein the progeny plant used in said genomic DNA extracting step is in the form of seeds or a young seedling, and the genomic DNA is extracted from the seeds or the young seedling.

4. The method for producing a sugarcane line according to claim **1**, wherein the progeny plant(s) produced using the selected plant as a parent plant for crossing is in the form of seeds or a young seedling.

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